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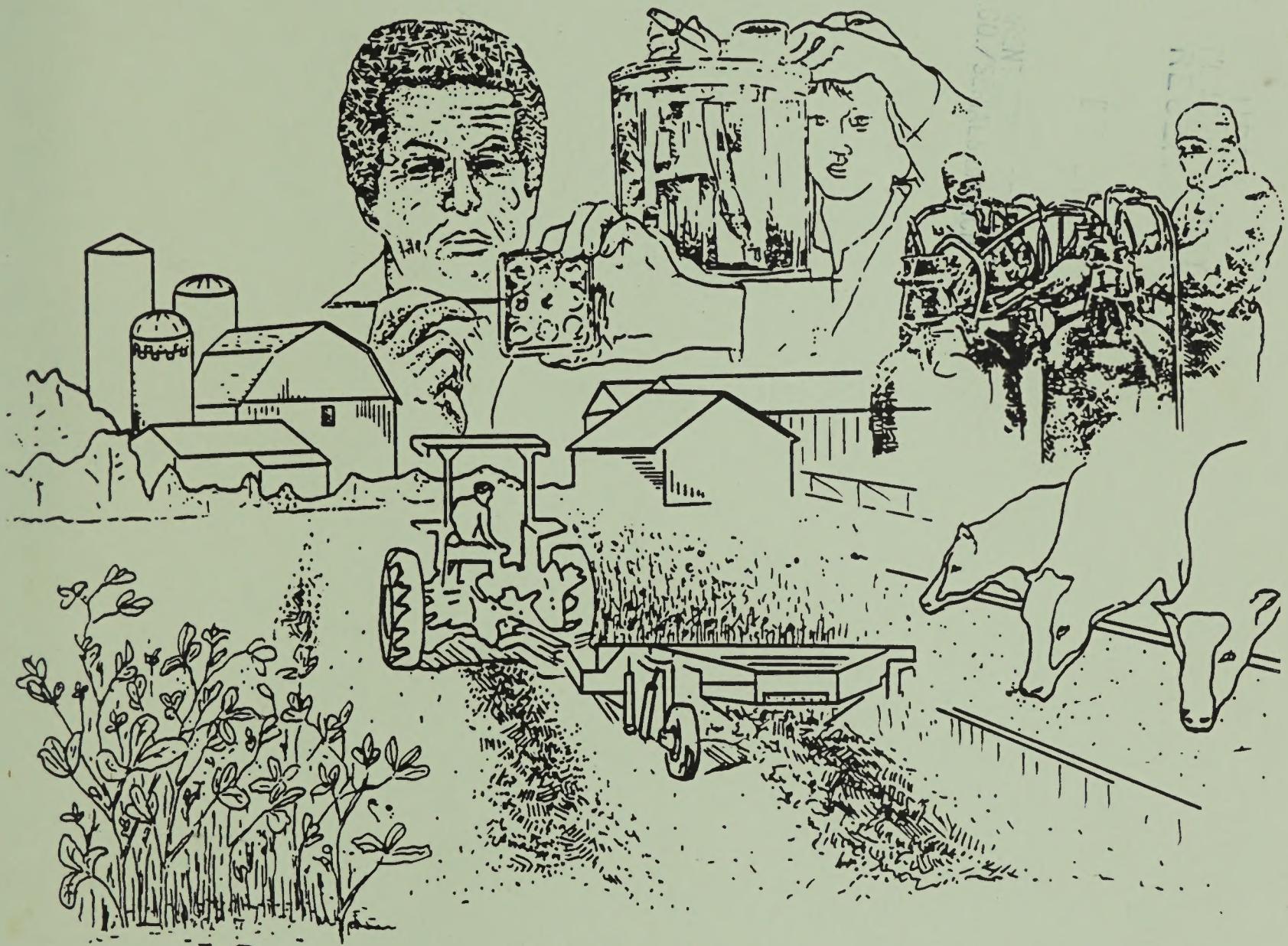
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U.S. DAIRY FORAGE RESEARCH CENTER

1988 RESEARCH SUMMARIES



U.S. Dairy Forage Research Center
1925 Linden Drive West
Madison, Wisconsin 53706



Agricultural
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United States
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March 1989

U.S. DAIRY FORAGE RESEARCH CENTER, USDA-ARS
Madison, WI 53706

Dear Reader:

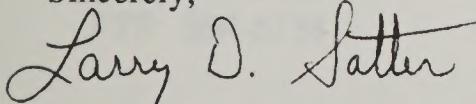
It is a pleasure to update our progress by bringing you these summaries of recent research. The U.S. Dairy Forage Research Center is a unique part of the national research program of the Agricultural Research Service, U.S. Department of Agriculture. The Center's mission is to build a knowledge and technology base for the dairy industry to fully exploit the use of forages in the production of milk. The Center has agricultural engineers, plant and soil scientists, microbiologists, ruminant nutritionists and a chemist working together to increase the efficiency of forage production and utilization by dairy farmers. We function in close cooperation with the Agricultural Experiment Stations of several states. The Center is located on the campus of the University of Wisconsin, Madison, and has "Cluster" locations in St. Paul, MN, Ames, IA, Columbia, MO, Wooster, OH, East Lansing, MI, University Park, PA and Ithaca, NY. The Center's research farm, with facilities for 300 milking cows, is located on 63 acres of USDA land on the banks of the Wisconsin River in Prairie du Sac, WI. An additional 1200 acres of adjacent land is utilized by the Center by agreement with the U.S. Department of the Army.

The Center was established in 1980 and has made steady growth since. At present there are eighteen scientists; ten at Madison, and one at each of six Cluster locations, and two at the St. Paul, Minnesota Cluster location. Dr. Paul Weimer came to Madison from DuPont this year to fill the microbiologist position, and Dr. John Ralph from the University of California-Berkeley to fill the chemist position. We are very pleased to have these two outstanding individuals join our staff.

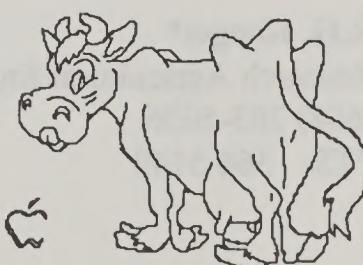
We are making a major effort to upgrade DAFOSYM (Dairy Forage Simulation Model developed by Dr. Al Rotz and his colleagues at the Michigan Cluster location) to a strategic decision making aid. We want to expand the crop model part of DAFOSYM to include year to year management effects on alfalfa growth and survival. We have hired Dr. Paul Wilkens, a recent graduate of Cornell, to assist in this development. Dr. Wilkens will start in April as a Research Assistant and will be located at the Michigan Cluster. More quantitative relationships between forage quality and milk production must also be established through further research to improve prediction capabilities of the model. We feel the efforts in this area will be very important in identifying ways to improve profitability of the dairy enterprise.

We are pleased and very proud of the way Center scientists from diverse disciplines interact and bring their collective insights to bear on the problems of forage production and utilization. This collection of research summaries illustrates the progress they are making in developing information to help dairy farmers utilize their resources more effectively. The research is intended to benefit dairy farmers and the consumers of dairy products.

Sincerely,



Larry D. Satter, Director
U.S. Dairy Forage Research Center



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Acknowledgments

Appreciation is expressed to Gloria Palmer for her interest and dedication to the task of typing and assembling of this annual research summary, and to Dr. John Ralph for assisting with the application of new equipment and software in this effort, a contribution that was far beyond the call of duty.

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MEASUREMENT OF SYMBIOTICALLY-FIXED NITROGEN IN SOIL SURROUNDING ALFALFA ROOTS

J.L. LORY, M.P. RUSSELLE and G.H. HEICHEL

Introduction

One mechanism for a legume such as alfalfa to contribute symbiotically-fixed nitrogen (SFN) to an agricultural system is by deposition of fixed N into the soil. Potential mechanisms of below-ground N loss from a legume to soil include: decay of roots and nodules, diffusion of ammonia from the nodules, exudation of nitrogenous compounds, and sloughing of root material. Contradictory results were obtained in early work quantifying loss of SFN to soil. In a series of experiments alfalfa released to soil 0 to 50% of the N fixed by the plant (Wilson, 1940). Recent work has shown that pigeon pea released as much SFN to the soil as remained in the tops (Poth et al. 1986), and that hydroponically-grown alfalfa lost 3% of its fixed N into the solution surrounding the roots. Presence of a significant pool of SFN in the soil surrounding roots would have important implications in how we view the role of legume-N in rotations and intercrops.

Materials and Methods

In this field study we quantified deposition of SFN in the rhizosphere of alfalfa plants using a soil ^{15}N dilution technique. Alfalfa plants were grown in a soil with a ^{15}N label incorporated into the organic matter. If deposition of SFN (at natural abundance) into the rhizosphere occurred, it would dilute the stable soil label. We compared deposition of fixed-N in soil surrounding the roots of effectively nodulated Saranac alfalfa with the increase in fixed-N in the soil surrounding ineffectively-nodulated Saranac-IN alfalfa. Because the Saranac-IN symbiosis does not fix N_2 , any apparent deposition of atmospheric-N would be due to non-symbiotic fixation.

The Hubbard sandy loam soil had been labeled initially with ^{15}N fertilizer in May 1979. In May 1987 the soil had an ^{15}N concentration of about 0.400 atom %. Ten-week old plants were transplanted into four paired microplots (effective versus ineffective) on May 10, 1987. The plots were fertilized with 0.400 atom % ^{15}N solution ($1:1 \text{ NH}_4\text{:NO}_3$) at the rate of 10 kg week^{-1} to insure sufficient growth of the ineffectively-nodulated plants.

At the third harvest in September 1987 7.5-cm diameter cores centered over single alfalfa plants were taken with a hydraulic probe to a depth of 75 cm. In the lab soil cores were dissected to provide samples of roots, nodules, bulk soil, rhizosphere soil (that soil that adhered to the roots), and nondosphere soil (that soil that adhered to the nodules). Meticulous care was taken to remove all root material from soil samples. Rhizosphere soil was rinsed from the roots with sodium phosphate buffer solution. The resulting soil solution was carefully strained to remove all visible root pieces. We obtained root and shoot dry mass and analyzed all soil and plant samples for N content and atom % ^{15}N .

Results and Discussion

The shoots and roots of effectively-nodulated plants obtained 76 and 59% of their N from fixation, respectively. Shoot dry matter yields were significantly greater for Saranac than Saranac-IN over the three harvests ($p<0.05$). This raised questions about the suitability of Saranac-IN as a control for Saranac. However total root dry matter yield was not significantly different. Figure 1 compares mean root dry mass yields ($n=12$) in four soil horizons. Only nodule mass differed significantly.

No significant differences were observed in the total N content or the atom % ^{15}N of the rhizosphere soil when effectively nodulated plants were compared with ineffectively-nodulated plants. A sensitivity analysis of the method demonstrated that SFN in the rhizosphere would need to exceed 3.5 kg ha^{-1} to be detected by the soil ^{15}N dilution technique, and 10.5 kg ha^{-1} to be detected by the difference in total-N technique. Consequently we concluded that no significant pool of SFN existed in the rhizosphere of these alfalfa plants at the time of harvest.

To properly interpret the results of this study, the limitations must be acknowledged. 1) Our method was not sensitive enough to detect SFN that may have been deposited in the bulk soil. 2) This was a point measurement of SFN in the rhizosphere, not a cumulative measurement. 3) SFN that was deposited in the soil but then taken up by the root would not be detected by this method. 4) This method was most likely to detect SFN in the rhizosphere at the time of harvest from diffusion of NH_3 from the nodules, exudation of nitrogenous compounds, and sloughing of root material from live roots. 5) SFN from the decay of roots and nodules was unlikely to be quantified in this experiment. Other experiments that do not have these limitations are being conducted.

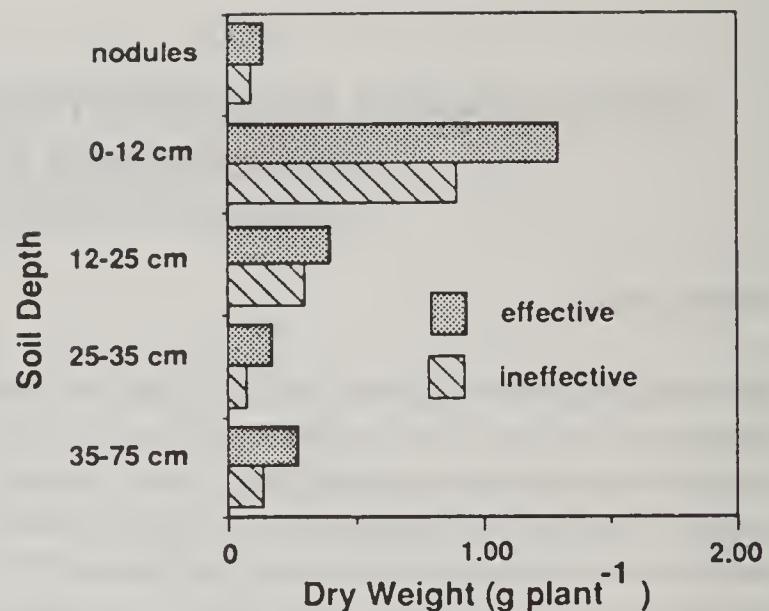


Figure 1. Root dry mass yields in four soil depths and total nodule dry mass yield. For all means $n=12$. Only nodules were significantly different at $p<0.05$.

ALFALFA CULTIVAR, AUTUMN HARVEST DATE, AND FUNGICIDE EFFECTS ON I. LEAF DISEASE DEVELOPMENT AND CHEMICAL COMPOSITION

D.J. DENEEN, R.P. WALGENBACH and C.R. GRAU

Introduction

Leaf diseases often develop on alfalfa during autumn in the upper Midwest. Most currently grown cultivars have little resistance to foliar disease, although some cultivars have moderate levels of resistance. Little information is available on autumn leaf disease affects on alfalfa chemical compo-

sition. Our objectives were to determine the influence of alfalfa cultivars, fungicide treatment and autumn cutting schedules on leaf disease development during autumn and chemical composition.

Materials and Methods

Plots of Funks G2852 and Apollo II alfalfa were seeded on 18 April, 1986 and harvested on or near 10 and 24 October 1986 and 1987. These were compared to uncut plots. Tilt fungicide was applied once the 2" third harvest regrowth 1986. Fungicides were switched in 1987 because we felt that multiple applications would improve leaf disease suppression. A combination of benomyl and mancozeb was applied at 10 day intervals beginning 21 August 1987. Herbage samples were visually evaluated for leaf disease. Stem and leaves were separated, weighed and analyzed for ADF, NDF, IVDMD and CP concentrations.

Results and Discussion

More favorable weather for leaf disease development occurred in Autumn 1986 than in 1987. Apollo II had more leaf disease (Table 1), and lower forage quality (Table 3) than did Funks G2852 during autumn 1986. But these cultivars did not differ for those measurements during autumn 1987. The 1986 fungicide treatment had little effect on leaf disease or forage quality measurements. But, the 1987 fungicide treatment reduced leaf disease ratings (Table 2) and increased autumn forage quality (Table 3). Autumn leaf disease reduced forage quality. Fungicide and cultivar suppression of leaf disease would be most beneficial in maintaining autumn forage quality where weather conditions favor leaf disease development. However, a more comprehensive understanding of environmental conditions in relation to pathogen populations is needed in order to better define economic thresholds and autumn leaf disease forecasting methods.

Table 1. Influence of Apollo II and Funks G2852 on portion of leaves diseased during autumn 1986 and 1987.

<u>disced</u> Cultivar 1987	Autumn Harvest	<u>Portion of leaves</u>	
		1986	%
Apollo II	10 Oct	17	44
Funks G2852	10 Oct	6	38
Apollo II	24 Oct	60	58
Funks G2852	24 Oct	27	50
LSD 0.05		4	8

Table 2. Influence of tilt applied in 1986 and mancozeb + benomyl applied in 1987 on portion of leaves diseased during autumn 1986 and 1987.

Fungicide	Autumn Harvest Date	<u>Portion of leaves diseased</u>	
		1986	1987
+	10 Oct	16	33
-	10 Oct	16	50
+	24 Oct	41	47
-	24 Oct	46	64
LSD 0.05		4	8

Table 3. Influence of propiconazole applied in 1986 and mancozeb plus benomyl applied in 1987 and autumn harvest date on concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), in vitro dry matter disappearance (IVDMD), and crude protein (CP) in alfalfa leaf, stem, and combined leaf and stem tissue.

Fungicide	Harvest Date ¹	NDF		ADF		ADL		IVDMD		CP	
		1986	1987	1986	1987	1986	1987	1986	1987	1986	1987
		g kg ⁻¹									
+	10 Oct	227	202	173	153	52	32	756	810	316	238
+	24 Oct	268	241	192	177	59	41	721	782	297	230
+	11 Nov	287	329	200	236	60	62	731	721	274	234
-	10 Oct	229	219	169	157	50	39	752	791	317	256
-	24 Oct	281	276	204	194	66	48	715	749	298	250
-	11 Nov	302	369	213	258	68	74	706	678	279	252
LSD 0.05		15	11	15	9	9	4	26	15	7	7
LSD 0.05											
between years		13		12		7		21		7	
+	10 Oct	494	544	420	457	90	98	674	618	154	112
+	24 Oct	537	551	447	461	101	98	637	593	129	104
+	11 Nov	551	633	460	516	106	118	622	539	118	102
-	10 Oct	506	538	426	445	93	97	667	600	155	110
-	24 Oct	535	578	448	481	101	106	641	576	133	104
-	11 Nov	548	663	459	534	106	126	624	502	122	103
LSD 0.05		14	25	13	19	4	5	10	30	6	6
LSD 0.05											
between year		19		16		4		20		6	
+	10 Oct	367	364	302	297	72	63	713	720	232	178
+	24 Oct	430	398	346	322	84	70	672	686	195	166
+	11 Nov	458	522	368	413	90	97	660	606	173	150
-	10 Oct	378	383	308	306	73	69	706	693	229	181
-	24 Oct	436	446	353	356	88	81	669	652	197	168
-	11 Nov	462	580	373	456	93	111	648	551	177	145
LSD 0.05		12	17	11	14	5	4	12	18	6	5
LSD 0.05											
between years		15		13		5		15		6	

¹ Alfalfa sampled on 13 and 27 October 1987 and 12 November 1987.

ALFALFA CULTIVAR, AUTUMN HARVEST DATE, AND FUNGICIDE EFFECTS ON II. DRY MATTER YIELDS AND STAND PERSISTENCE

D.J. DENEEN, R.P., WALGENBACH AND C.R. GRAU

Introduction

Leaf diseases may reduce the plant's photosynthetic capacity resulting in decreased carbohydrate synthesis, translation for root storage, and/or plant growth. Most currently grown cultivars have little resistance to foliar disease, although some cultivars have moderate levels of resistance. Little information is available on autumn leaf disease effects on alfalfa growth and stand productivity. Our objectives were to determine the influence of alfalfa cultivars, fungicide treatment and autumn harvest schedules on forage yield and root TNC content, and on spring regrowth vigor and stand persistence.

Materials and Methods

Plots of Funks G2852 and Apollo II were seeded on 18 April, 1986 and harvested on or near 10 and 24 October 1986 and 1987. These were compared to uncut plots. Tilt fungicide was applied once to 2" third harvest regrowth in 1986. Fungicides were switched in 1987 because we felt that multiple applications would improve leaf disease suppression. A combination of benomyl and mancozeb was applied at 10 day intervals beginning 21 August 1987. Herbage samples were visually evaluated for leaf disease. Roots were analyzed for total nonstructural carbohydrate (TNC) content. Visual root discoloration ratings were made during late summer 1987 and 1988.

Results and Discussion

More favorable weather for leaf disease development occurred in autumn 1986 than in 1987. Autumn leaf disease was suppressed, while DM yield was increased by a more resistant cultivar in 1986, and by fungicide in 1987. (Table 1) Dry matter yield declined 20% between 10 and 24 October, 1986 compared to 5% between 13 and 27 October, 1987.

Harvesting alfalfa during autumn increased the extent of crown and root injury and reduced the following year's DM yield (Table 2) and final stand count. Researchers need to better evaluate the potential risks that autumn alfalfa harvest has on stand productivity and persistence in relation to geographic location, autumn and winter weather patterns and management practices.

Table 1. Influence of cultivar, harvest date and fungicide on autumn dry matter (DM) yield in 1986 and 1987.

Cultivar	DM YIELD		Harvest Date	DM YIELD		Fungicide	DM YIELD	
	1986	1987		1986	1987		1986	1987
Apollo II	1344	1958	10 Oct	1739	2026	+	1617	2138
Funks G2852	1774	1991	24 Oct	1397	1923	-	1502	1811
Significance	**	NS		**	*		*	**

*,**Significant at the 0.05 and 0.01 probability levels respectively.

Table 2. Influence of 1986 and 1987 autumn harvest date on 1987 and 1988 root discoloration ratings, dry matter (DM) yield and final starch count.

Harvest Date	Root Discoloration Rating		1987 DM Yield			1988 DM Yield			Final Stand Count		
	1987	1988	6/2	7/7	8/12	6/2	7/6	8/8			
			kg ha ⁻¹								
10 Oct	1.9	2.5	4052	3841	3645	3614	3373	1945	1709	49.5	
24 Oct	1.7	2.3	4233	3832	3709	3982	3637	2121	1950	53.4	
Uncut	1.3	1.6	5089	4047	3722	4402	3686	2192	2143	68.1	
LSD 0.05	0.2	0.2	283	175	NS	257	NS	NS	208	3.1	

FORAGE QUALITY AND CHEMICAL COMPOSITION

ALKALI-TREATED ALFALFA AND ORCHARDGRASS: EFFECT ON COMPOSITION AND IN SITU DEGRADATION OF CELL WALL COMPONENTS

C.J. CANALE, S.M. ABRAMS, G.A. VARGA and L.M. MULLER

Introduction

Alkaline treatment of forage has been shown to alter forage composition and disrupt barriers that limit cell wall utilization. In a previous study with an alfalfa-orchardgrass sward, alkali treatment reduced the amount of indigestible fiber and stimulated increased feed intake, milk production, and fiber digestibility in early lactation dairy cows. The objective of this study was to determine the effect of varying levels of alkali treatment on content and degradation of cell wall components of alfalfa and orchardgrass at two stages of maturity.

Methods and Materials

Three field replicates of first-cutting alfalfa (bud and mid-bloom) and orchardgrass (pre-head and head) were treated at harvest with no solution (NS), 2, 4, 6, or 8 g NaOH/100g forage DM. Forages were hand cut, hand-sprayed, and sun-cured as hay. Disappearance of NDF and cell wall phenolic acids and neutral sugars were determined by polyester bag technique. Bags containing 8 g of forage (4-mm) were placed in the rumen of 2 fistulated cows and incubated for 6, 12, 24, 36, 60, and 72 h. First order kinetics adequately described extent of NDF and phenolic acid disappearance. Extent of neutral sugar digestion was considered to be the amount digested at 72 h. Degradation data are presented here.

Results and Discussion

Alkali treatment increased potentially digestible NDF in all forages, except immature orchardgrass, which contained NDF that was almost completely digestible (Table 1). Degradation of FER and PCA was increased in immature and mature orchardgrass, respectively, as a result of NaOH treatment. Phenolic acids were not detected in alfalfa. Alkali-treated orchardgrass had greater disappearance (72 h) of xylose and glucose compared to untreated orchardgrass, regardless of maturity. Alkali treatment increased extent of xylose digestion in immature alfalfa. Concentration of PCA and FER in orchardgrass cell wall was not affected by NaOH treatment ($P>.10$). Except for reduced galactose in mature orchardgrass, NaOH treatment had no effect on the composition of cell wall neutral sugars of any forage, regardless of maturity. Patterns of change in degradation of neutral sugars with increasing amounts of alkali were similar among the three sugars in orchardgrass. In alfalfa, alkali treatment had little effect on arabinose which was highly degradable in untreated alfalfa. Overall, consistent patterns of response to alkali treatment between degradation of NDF and degradation of either phenolic acid monomers or neutral sugars were not observed across species and maturities.

Table 1. Effect of alkali treatment on degradation of cell wall components from orchardgrass and alfalfa hay.

Forage	Maturity	NaOH (% DM)	NDF1	PCA2	FER3 % Degradation	ARAB4	XYL5	GLU6
Orchard- grass	Immature	NS	91.65	76.82	87.56	90.41	79.50	84.68
		2	91.23	78.44	91.28	96.21	89.58	91.88
		4	90.47	79.98	91.85	95.57	89.55	90.79
		6	91.66	79.37	99.00	95.27	84.86	88.12
		8	93.60	79.81	95.63	94.75	85.98	88.58
	Mature	SE	2.26	6.29	3.18	1.17	2.15	.83
		P	ns	ns	***	**	**	****
		NS	70.23	53.92	81.31	79.84	58.89	56.65
		2	71.17	67.99	80.04	83.17	60.45	53.07
		4	78.67	63.59	89.48	93.48	77.45	76.21
Alfalfa	Immature	6	85.65	62.25	81.79	79.54	67.79	63.36
		8	90.77	73.92	94.37	89.65	82.05	80.33
		SE	1.89	3.86	.81	5.65	5.27	6.02
		P	*	***	ns	ns	***	***
		NS	54.94			100.00	45.34	62.49
	Mature	2	64.08			100.00	51.03	67.65
		4	69.99			95.39	42.07	67.39
		6	73.75			100.00	47.81	62.87
		8	71.50			96.02	56.52	73.42
		SE	1.54			2.36	2.89	3.69
		P	*			ns	***	ns
	NS	46.79			100.00	35.09	52.97	
		2	53.29			100.00	44.95	59.29
		4	54.05			94.88	27.79	50.65
		6	55.25			94.07	64.85	70.71
		8	59.41			100.00	42.72	57.81
	SE	1.20			3.04	10.93	9.37	
		P	*		ns	ns	ns	

1 Potentially digestible NDF.

2 Potentially digestible p-coumaric acid.

3 Potentially digestible ferulic acid.

4 Extent of arabinose digestion at 72h.

5 Extent of xylose digestion at 72h.

6 Extent of glucose digestion at 72h.

* Linear NaOH effect (P<.01).

** Quadratic NaOH effect (P<.05).

*** Linear NaOH effect (P<.10).

**** Quadratic NaOH effect (P<.10).

DIGESTIBILITY OF CELL-WALL CARBOHYDRATES FROM GRASS AND LEGUME STEMS

D. R. BUXTON and M.R. BRASCHE

Introduction

Comprised largely of cellulose, hemicellulose, and lignin, plant cell walls are responsible for most of the variation in digestibility that occurs in herbage dry matter. As plants mature, significant changes occur in cell-wall relationships, mostly because of the increase in lignification. This study was conducted to determine the effect of plant aging on digestibility of cellulose and hemicellulose and their influence on cell-wall digestibility.

Materials and Methods

The basal 20 cm of immature and mature stems of alfalfa, birdsfoot trefoil, smooth bromegrass, and orchardgrass were collected from a field planting with four replicates. Samples were incubated in buffered rumen fluid for 0, 6, 12, 24, 48 and 72 h. Cellulose and hemicellulose were calculated as the differences between acid-detergent fiber (ADF) and lignin-plus-ash concentrations, and between neutral-detergent fiber (NDF) concentrations, respectively. Data for NDF, cellulose, and hemicellulose were fitted with a first-order, nonlinear digestion model.

Results and Discussion

Stem age had little effect on the apparent lag before digestion of NDF(3.6 h) or hemicellulose (2.6 h), but increased the lag before cellulose digestion from 2.2 to 4.3 h. Also, stem age decreased the concentration of potentially digestible NDF from 335 to 177 g kg⁻¹ dry matter in grass stems, but only from 228 to 202 g kg⁻¹ dry matter in legume stems.

The concentrations of potentially digestible cellulose (171 g kg⁻¹ dry matter) and hemicellulose (63 g kg⁻¹ dry matter) were not statistically influenced by stem age in legumes. In grass stems, however, potentially digestible cellulose decreased from 212 to 99 g kg⁻¹ dry matter and potentially digestible hemicellulose decreased from 147 to 97 g kg⁻¹ dry matter. The large decrease in grass stems occurred because indigestible cellulose rose from 186 to 295 g kg⁻¹ dry matter. Indigestible hemicellulose in grasses was unaffected by stem age (136 g kg⁻¹ dry matter). In legume stems, indigestible cellulose increased from 216 to 282 g kg⁻¹ dry matter with age.

Rate of digestion of potentially digestible NDF, cellulose, and hemicellulose were generally not affected by stem age in legumes, but in grass stems, NDF rate of digestion was reduced by a large decrease in hemicellulose rate of digestion with stem age (Table 1).

Table 1. Digestion rate of potentially digestible cell-wall components.

Species	NDF		Cellulose		Hemicellulose	
	Immature	Mature	Immature	Mature	Immature	Mature
..... ^{h⁻¹}						
Alfalfa	0.059	0.058	0.066	0.076	0.046	0.028
Birdsfoot Trefoil	0.062	0.060	0.045*	0.063	0.047	0.082
Legume mean	0.061	0.059	0.056	0.070	0.047	0.055
Smooth Bromegrass	0.076*	0.040	0.061	0.037	0.099	0.080
Orchardgrass	0.076*	0.043	0.050	0.079	0.193*	0.040
Grass mean	0.076**	0.042	0.060	0.058	0.146*	0.060

*,** P= 0.05 and 0.01, respectively, for maturity comparison of a cell-wall component.

CELL-WALL COMPONENTS IN DIVERGENT GERMPLASMS OF FOUR PERENNIAL GRASS SPECIES

D.R. BUXTON

Introduction

Much of the variability in digestibility of herbage is closely associated with concentrations of neutral-detergent fiber (NDF) and cell-wall (CW) components. This study was conducted to determine whether concentration differences existed for NDF and CW components in divergent germplasms of four perennial grass species and whether they could account for reported variation in digestibility.

Materials and Methods

‘Napier’ and ‘Orion’ orchardgrass, ‘Barton’ and ‘Rebound’ smooth bromegrass, germplasm of reed canarygrass selected for high (HSLW) or low (LSLW) specific leaf weight, and germplasm of tall fescue selected for high (HLER) or low (LLER) rate of leaf-area expansion were studied. At several spring harvests, total herbage and plant parts were assayed for NDF and CW components.

Results and Discussion

Although there were large differences in tiller size, NDF concentration of herbage and plant parts did not vary consistently between the germplasms of reed canarygrass or tall fescue. Thus, there was no evidence that selecting for fast growth rate and large tillers affected NDF concentration or concentrations of CW components.

In orchardgrass, NDF concentration was only 3% greater in Napier than in Orion herbage. Lignin concentration in the CW, however, was 24% greater in herbage of Napier than in that of Orion (Table 1). Average NDF concentration was 5% greater in herbage and stems of Barton than in those of Rebound smooth bromegrass. Furthermore, stems of Barton had 5% greater CW-lignin concentration than those of Rebound (Table 1).

Cultivar variation for herbage digestibility of orchardgrass was an inverse function of CW-lignin concentration. In smooth bromegrass, cultivar differences were influenced equally by variation in NDF concentration and by variation in CW-lignin concentration. The results suggest that it should be possible to breed for low CW-lignin concentration and associated high CW (or fiber) digestibility. This may be a means of both increasing the energy available to ruminants from forages and maintaining the fiber content necessary for proper rumen function.

Table 1. Lignin concentration in total herbage cell walls of grasses at various sampling dates.

Date	Orchardgrass		Smooth bromegrass		Reed canarygrass		Tall fescue		Entry LSD(0.05)+
	Napier	Orion	Barton	Rebound	LSLW	HSLW	LLER	HLE	
.....g kg ⁻¹ NDF.....									
Year 1									
19 May	44.6**	32.5	43.7	49.5*	42.4	41.4	38.0	44.0*	5.09
16 June	67.2**	49.3	70.4	66.8	58.6	59.6	61.1	61.0	5.50
30 June	71.2**	53.2	82.3**	73.0	67.0	68.6	67.4	64.4	5.30
Mean	61.0**	45.0	65.5	63.1	56.0	56.6	55.5	56.5	2.80
Year 2									
10 May	39.9	39.8	42.2	54.3*	45.7*	39.3	20.1	19.5	5.30
24 May	39.8**	29.5	39.6	45.2	39.5	38.5	26.3	22.4	5.88
7 June	57.1**	39.7	53.0	52.7	47.0	46.9	39.9	39.3	4.65
21 June	74.0**	59.5	72.5	69.8	66.9	65.6	58.0	54.1	4.65
5 July	78.3**	70.0	80.6	78.5	75.0	72.6	60.8	61.0	4.16
Mean	57.8**	47.7	57.6	60.1	54.8	52.5	41.0	39.3	2.73

*,** Indicates significant differences at the 0.05 and 0.01 probability levels, respectively, between the two entries within a species based upon orthogonal comparisons.

+ LSD for comparing all entries within a sampling date.

FORAGE QUALITY OF PLANT PARTS OF PERENNIAL GRASSES AND RELATIONSHIP TO PHENOLOGY

D. R. BUXTON and G. C. MARTEN

Introduction

Improving forage quality, while maintaining or increasing herbage yield, is a desirable goal. This can be simplified if interrelationships of forage quality among plant parts and the effect on forage quality of morphological characters associated with yield are better understood. Plants with large tillers and plant parts have high yield potential, but may be of low forage quality because of high concentrations of lignin and other structural constituents needed to support large plant parts. Furthermore, cumulative effects of environment and herbage age are integrated through growth and development and expressed in growth stage and forage quality. The usefulness of a growth-stage-classification system can be determined by comparing it to forage quality. Growth-stage-classification systems used for perennial grasses have lacked the necessary uniformity and precision for this purpose. Simon and Park recently proposed a quantitative classification system. Our objectives in

this study were to determine the (i) interrelationships of forage quality among grass plant parts of germplasm that differed in forage quality of total herbage, (ii) influence of selecting for divergent morphological form on forage quality, and (iii) association among plant morphological stage, forage quality and growing degree days (GDD).

Materials and Methods

These relationships were studied in 'Napier' and 'Orion' orchardgrass, 'Barton' and 'Rebound' smooth bromegrass, germplasm of reed canarygrass selected for either high or low specific leaf weight, and germplasm of tall fescue selected for either high or low rate of leaf-area expansion. The experiment was conducted during spring growth during two years in Iowa.

Results and Discussion

Morphological stage determined by the Simon and Park method was closely related to accumulated GDD by using a 5°C base ($r^2 = 0.97$). Within each year, decline in herbage in vitro digestible dry matter (IVDDM) and crude

protein (CP) concentration was closely related to calendar day (Fig. 1), GDD, and morphological stage, although least to morphological stage. For morphological staging systems to more accurately predict forage quality, the proportion of reproductive and vegetative tillers in swards will need to be considered as well as their development. Larger cultivar differences in IVDDM occurred within stems of orchardgrass (24%) than within leaves (7%). In smooth bromegrass, Rebound stems were 12% more digestible than those of Barton, but Barton leaves were 6% more digestible than Rebound leaves. There were few genetic differences in CP concentration within any species and no consistent differences in IVDDM within the reed canarygrass or tall fescue germplasms. Thus, cultivar variation for IVDDM was not uniform among plant parts, and selecting grass plants on the basis of specific leaf weight or leaf-area expansion rate seems to have little effect on forage quality.

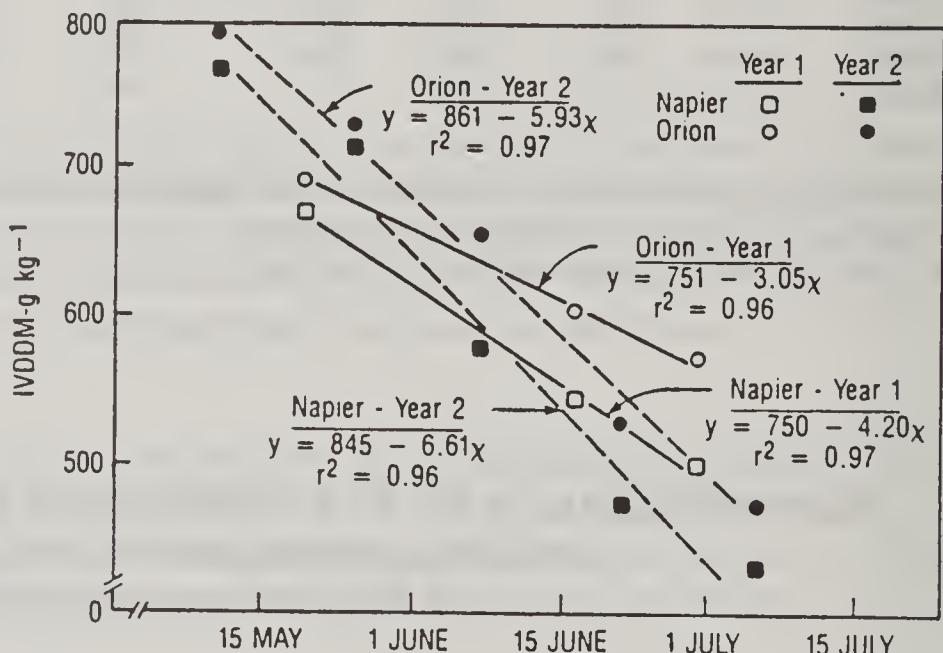


Figure 1. *In vitro digestible dry matter (IVDDM) in total herbage of orchardgrass cultivars.*

ERRORS IN DATA PREDICTED BY NEAR INFRARED REFLECTANCE SPECTROSCOPY RELATIVE TO FORAGE QUALITY

D.R. BUXTON and D.R. MERTENS

Introduction

Near infrared reflectance spectroscopy (NIRS) is rapidly being accepted for routine prediction of forage quality and chemical constituents. It is commonly stated to be as accurate or more accurate than conventional methods as long as the instrument is properly calibrated. In our work with samples for which numerous forage quality and chemical constituents had been determined by conventional methods, we discovered that deviations between conventionally determined analyses and those predicted by using NIRS, calibrated with the same samples, was not random. Instead, much of the deviation could be explained by the statistical model used in the design of the experiment. We report here only results for in vitro true digestibility (IVTD), neutral-detergent fiber (NDF), and lignin, but similar results were obtained for cell-wall neutral sugars and nitrobenzene oxidation products of core lignin.

Materials and Methods

A total of 108 samples of immature and mature stem bases of grass and legume species and cultivars were studied. Samples were collected from a replicated field study, but data only for Year 2 are reported here. Predicted NIRS values were subtracted from the values determined by conventional procedures. These deviations were analyzed by analysis of variance (ANOVA).

Results and Discussion

The accuracy with which we are able to predict IVTD, NDF, and lignin concentrations compares favorably with that achieved by others using NIRS (Table 1). The ANOVA revealed that several significant deviations were accounted for by the statistical model associated with the experimental design. This suggests that some treatment differences observed when NIRS is used to predict composition (in research trials) is related to varying accuracy of NIRS among sources of variation. Some of the deviation occurred because we predicted both grasses and legumes values with the same equation. Often, however, deviations for field replicates and cultivars within species were significantly different (Tables 1 and 2). Even within species, our results point to the need for caution in use of NIRS to evaluate management and germplasm effects on forage quality. It is imperative that prediction equations have low SE and that results from NIRS data be checked routinely with conventional analyses.

Table 1. Squared coefficients of multiple determination (R^2) and standard error (SE) from regression of known quality values on NIRS values, and mean squares for combined harvests in Year 2.

	IVTD	NDF	Lignin
R^2	0.95	0.96	0.98
SE - g kg ⁻¹	31.8	19.2	7.6
<u>Mean squares</u>			
Replicate	2256*	316	289**
Entry	1166	177	78
Error (a)	643	249	44
Maturity	466	50	29
Entry x maturity	2983**	369	92
Legume vs grass	13037**	350	390**
Brome vs orchard	100	410	135
Barton vs Rebound	102	35	14
Napier vs Orion	5620**	321	19
RC vs trefoil, alfalfa	607	301	3
Trefoil vs alfalfa	4322	292	172
Magnum vs Spredor 2	57	1159**	4
Empire vs Viking	21	82	1
Error (b)	584	156	47

*,** Significant at 0.05 and 0.01 levels of probability, respectively.

Table 2. Known values minus NIRS values for in vitro true digestibility (IVTD), neutral-detergent fiber (NDF), and lignin.

Species/cultivar	IVTD		NDF		Lignin	
	Immature	Mature	Immature	Mature	Immature	Mature
.....g kg ⁻¹ dry matter.....						
Smooth bromegrass						
Barton	1.5	-25.0	-2.8	-5.2	-8.3	4.2
Rebound	25.8	-10.9	-5.1	-1.5	-7.3	1.4
Mean	13.7	-17.9	-4.0	-3.4	-7.8	2.8
Orchardgrass						
Napier	-8.8	-10.0	6.0	1.2	4.7	4.9
Orion	32.4	-43.8	15.7	-7.0	0.9	5.4
Mean	11.8	-26.9	10.9	-2.9	2.8	5.2
Grass Mean	12.8	-22.4	3.4	-3.2	-2.5	4.0
Alfalfa						
Magnum	-7.0	-18.9	-19.6	3.0	-3.4	-1.0
Spredor 2	-5.6	-9.9	7.0	-4.4	2.1	2.6
Mean	-6.3	-14.4	-6.3	-0.7	-0.7	0.7
Birdsfoot trefoil						
Empire	-11.1	29.5	6.8	-4.2	1.2	-7.0
Viking	3.0	39.0	-5.8	-7.7	0.8	-6.6
Mean	-4.1	34.3	0.5	-6.0	1.0	-6.8
Red clover						
Arlington	-7.6	27.0	-12.6	0.6	-2.1	-4.0
Legume Mean	-5.7	13.4	-4.8	-2.6	-0.3	-3.2

FORAGE QUALITY OF ALFALFA AS AffECTED BY POTATO LEAFHOPPER FEEDING

S.H. HUTCHINS, D.R. BUXTON, and L.P. PEDIGO

Introduction

The potato leafhopper (PLH) has been identified as one of the most severe alfalfa pests in the North Central USA, and is frequently cited as limiting the production of alfalfa. The PLH feeds by inserting its piercing-sucking mouthparts into the phloem elements and extracting plant juices. Injury to the plant is believed to result from destruction and clogging of phloem tissue after repeated insertion of the stylet. Little information exists regarding its effect on forage quality.

Materials and Methods

Three field trials were conducted in 1984 and 1985 to determine the consequence of PLH feeding on chemical composition and nutritional quality of alfalfa stem and leaf components. A factorial arrangement of four densities of PLH adults (0, 50, 100, 200 m⁻²) and two infestation periods (1 and 14 d after first harvest) were arranged in a randomized complete-block design with four replicates. A split-plot in time was superimposed with subplots representing weekly plant samples for assays of digestibility, neutral detergent fiber (NDF), and crude protein.

Table 1. Effect of various infestation periods and densities of potato leafhopper (PLH) on herbage, stem, and leaf in vitro digestibility of alfalfa over three field trials.

Infest Period	PLH Density	Herbage IVDDM			Stem IVDDM			Leaf IVDDM		
		14	28	42	14	28	42	14	28	42
no. m ⁻²										g kg ⁻¹
Early (A)	0	738	730	661	737	625	563	818	773	744
	50	753	742	664	748	647	569	808	776	746
	100	741	715	664	754	658	554	800	783	726
	200	740	717	655	759	643	578	800	787	742
Late (B)	0	736	726	660	748	649	572	818	760	725
	50	750	712	677	749	647	558	806	775	733
	100	762	722	659	737	655	567	806	791	722
	200	736	727	669	747	650	589	820	785	750
Contrasts: +										
A: 0 vs. 50 to 200	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
A: 0 to 100 vs 200	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
A: 0 vs 50	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
B: 0 vs 50 to 200	NS	NS	NS	NS	NS	NS	NS	NS	**	NS
B: 0 to 100 vs 200	NS	ND	NS	NS	NS	**	NS	NS	**	NS
B: 0 vs 50	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
A vs B: 50 to 200	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

+E values for contrasts determined to be significant (*, P=0.05), highly significant (**, P=.01), or not significant (NS).

Results and Discussion

Total herbage in vitro digestibility did not differ among plots (Table 1). Digestibility of the stem component was actually enhanced (up to 3.0%) by severe PLH feeding. The leaf component was also slightly higher in digestibility (as much as 2.2%) under extreme PLH-induced stress. Total herbage NDF was largely unaffected at harvest by PLH-induced injury. Leaf proteins were reduced

(ca. 8.2%) in most infested plots, but stem proteins were maintained or even enhanced (up to 9.1%) with increasing levels of PLH-induced injury. Comparisons of chlorotic versus nonchlorotic leaves suggested that visible symptoms of PLH feeding did not necessarily indicate differences in chemical composition of the forage. This study indicates that pest management programs for PLH should be based primarily on reductions in biomass or nutrient yield, rather than on absolute quality reductions per biomass unit.

DIGESTION KINETICS OF ORCHARDGRASS RELATED TO MORPHOLOGY, PARTICLE SIZE, AND MATURITY GROUP

E.M. LENTZ and D.R. BUXTON

Introduction

A large range in morphological traits of orchardgrass had only small effects on in vitro digestible dry matter (IVDDM) in a previous study. We wondered whether grinding to a 1-mm particle size limited the effect of morphological differences on IVDDM and if more pronounced morphological effects would be observed with a larger particle size. To answer this question, research was conducted to investigate relationships among particle size, morphological traits, and in vitro digestion kinetics within two maturity groups of orchardgrass. By using digestion kinetics, we could determine which parameter of digestibility was most affected by particle size and morphological traits.

Materials and Methods

Three replicates of eight clones were selected from two maturity groups — four clones in each maturity group consisting of plants with long blades, short blades, narrow blades, and wide blades. Leaf data were obtained from regrowth material in the field and greenhouse, and stem data from spring growth in the field. Plant material was ground to pass a 8-mm screen; 50% of this material was then ground to pass a 1-mm screen. Plant material was digested in vitro with rumen liquor for 0, 6, 12, 24, 48, and 72 h. Following incubation, fiber was extracted by neutral detergent. These data were fitted with a first order, nonlinear digestion model that estimated lag time before digestion, digestion rate of potentially digestible cell wall, potentially digestible cell wall (PDCW) concentration, and indigestible residue (IR) concentration. Results were similar for field and greenhouse plants, so data for regrowth were pooled between the two growth environments.

Results and Discussion

The morphological traits had little effect on digestion kinetics regardless of whether the plants were ground to 1-mm or 8-mm particles. Particle size affected in vitro digestion kinetics. The lag was longer and the digestion rate was slower for 8-mm particles than for 1-mm particles (Table 1). The 8-mm particles also had a greater PDCW concentration than the 1-mm group. Incomplete release of cell solubles from the 8-mm particles probably accounts for this difference because the amount of IR with the two particle sizes was similar in leaves, and only a small difference occurred in stems.

Maturity had a greater effect on digestion kinetics of stems than of leaves (Table 2). The digestion rate was faster and the PDCW fraction was greater in the late group than in the early group. The largest differences were observed in the IR concentration. It was g kg^{-1} less in the late group than in the early maturity group. Maturity did not affect in vitro digestion kinetics for leaves except for IR concentration and this difference was quite small.

Table 1. The means of 1-mm and 8-mm particle size (PS) groups for digestion lag, digestion rate of potentially digestible cell wall, potentially digestible cell wall (PDCW) concentration, and indigestible residue (IR) concentration in regrowth leaves and spring stems of orchardgrass.

Tissue	PS	Lag	Rate	PDCW	IR
Leaves	mm	—h-	—h ⁻¹ —	—g kg ⁻¹ DM—	
	1	2.99	0.0902	452	164
	**		**	**	
Stems	8	4.91	0.0648	598	165
	1	4.29	0.0798	342	363
	**		**	**	
	8	5.68	0.0598	411	395

**Significant at 0.01 level between particle size.+ DM is dry matter.

Table 2. The means of early and late maturity groups for digestion lag, digestion rate of potentially digestible cell wall, potentially digestible cell wall (PDCW) concentrations, and indigestible residue (IR) concentration in regrowth leaves and spring stems of orchardgrass.

Tissue	Maturity	Lag	Rate	PDCW	IR
Leaves	Early	—h-	—h ⁻¹ —	—g kg ⁻¹ DM—	
	Late	3.81	0.0763	520	174
Stems	Early	4.09	0.0787	530	155
	Late	5.23	0.0677	333	453
		**	**	**	
		4.75	0.0867	419	304

** Significant at 0.01 level between maturity groups.
+ DM is dry matter.

FORAGE QUALITY OF ORCHARDGRASS IN RELATION TO MORPHOLOGICAL TRAITS

E.M. LENTZ and D.R. BUXTON

Introduction

Morphological traits vary greatly among genotypes of orchardgrass — such as growth habit, leaf characteristics, stem development, and seed yield. In addition, orchardgrass has a wide range of maturity, which has a large effect on its forage quality. We wanted to determine the effects of morphological traits and maturity on digestibility. To determine these effects research was conducted on orchardgrass with several divergent morphological traits from two maturity groups.

Materials and Methods

Three replicates of forty orchardgrass genotypes from two maturity groups were established in spaced plantings. Each maturity group consisted of two genotypes selected for divergent growth habit, vegetative tiller number, seed size, blade width, and blade length. Genotypes were harvested at approximately 8 cm on the same date from spring growth and summer regrowth. The material was separated into plant parts before determining in vitro digestible dry matter (IVDDM). These parts consisted of fertile blades, fertile stems (including sheaths), vegetative blades, and vegetative sheaths for spring harvest and vegetative parts for the summer harvest.

Results and Discussion

Plants selected for wide blades were frequently more digestible than plants selected for narrow blades (Table 1). Differences were more obvious in the spring than in summer growth. The other four morphological traits had little to mixed effects on digestibility of orchardgrass. Most of the differences in digestibility occurred in the fertile parts and the least in the vegetative parts. Morphological traits seemed to have little effect on digestibility in the summer regrowth harvest.

Maturity affected digestibility. The plants in the late maturity group had higher IVDDM values of each plant part in both spring and summer harvests. Fertile stems had the most variation between maturity groups. The IVDDM means over both spring harvests were 17% higher for the fertile stems of the late group than the early group. The effects of maturity on the other parts from the spring harvests and the summer harvests were approximately 2.5% greater IVDDM for the late maturity group.

Maturity and panicle length were negatively correlated with digestibility for all parts of the spring harvests. Long panicle lengths may have had more structural carbohydrates, thus leading to lower digestibility. Stem width was positively correlated with IVDDM in the parts of the spring harvests. This correlation suggests that plants with wide stems had a greater amount of sheath material than plants with narrow stems, possibly diluting digestibility effects of the stem tissue.

In summary, plants selected for wide blades were frequently higher in IVDDM than plants selected for narrow blades. The other morphological traits had mixed effects on digestibility. Plant parts of the late maturity group were higher in IVDDM than the early group at a common harvest date. Stem

width was positively correlated, and maturity and panicle length were negatively correlated with IVDDM of plant parts in spring harvests.

Table 1. In vitro digestible dry matter means for fertile and vegetative tiller parts of orchardgrass clones with diverging blade widths from initial spring growth and summer regrowth.

Plant Parts	Blade Types	
	Narrow	Wide
.....g kg ⁻¹ DM+.....		
Fertile Blades		
Spring 1986	653	682**
Spring 1987	644	656
Fertile Stems		
Spring 1986	578	645**
Spring 1987	587	625
Vegetative Blades		
Spring 1986	667	667
Spring 1987	640	661**
Summer 1986	654	648
Vegetative Sheaths		
Spring 1986	670	672
Spring 1987	681	717**
Summer 1986	670	677

**Significant at 0.01 level of probability.

+ DM is dry matter.

HEMICELLULOSIC FRACTION OF ALFALFA STEMS

R.D. HATFIELD

Introduction

In most forage plants the polysaccharides that make up the cell wall fraction, hemicellulose, account for 30-40% of the total matrix. They represent a significant amount of potential energy for ruminants though their close association with microfibrils may limit cellulose degradation. During maturation the utilization of structural polysaccharides decline and appears to be correlated with increased lignification. Evidence suggests that lignin is covalently linked to xylans, although the specific linkage type has not been identified. This project was undertaken to determine what changes occur in the hemicellulosic fraction during the maturation of alfalfa stems.

Materials and Methods

Alfalfa (*Medicago sativa*) plants were grown in a greenhouse under high pressure sodium lamps with a 14/10 day/night light regime. Plants were harvested at the bud stage, divided into apical nodes

(AN, top 2.5-3.0 cm), upper nodes (UN, 7-8 nodes) and lower nodes (LN, bottom 7-8 nodes) and freeze dried. Stems were separated from leaves and petioles and cut into 0.5-1.0 cm lengths before homogenizing in 20 mM sodium phosphate buffer (pH 7.0). The disrupted stem cells were collected on a filter screen system washed with 50 mM NaCl, acetone, and chloroform:methanol (2:1). Isolated cell walls were reduced to an average size of 500-750 microns by jar milling. Starch contamination was removed by α amylase and amyloglucosidase treatment. Pectins and lignin were removed by sequential ammonium oxalate and acidic chlorite extractions.

Hemicellulosic polysaccharides were extracted using a step gradient of KOH (0.25M, 0.5M, 1.0M, 4.0M). Each solution contained 10mM NaBH₄ to prevent loss due to reducing end peeling. Isolated polysaccharides were put on ice and neutralized with glacial acetic acid before dialysis. Samples were removed for total sugar, total uronics, and neutral sugar analysis before freeze drying the remaining dialyzed polysaccharides. The cell wall residue was subjected to total hydrolysis (incubation in 72% H₂SO₄ for 2.5 hours, dilution to 1.6M, and incubation at 100° C for 2.5 h) to assess efficiency of hemicellulose extraction.

Results and Discussion

The amounts of hemicellulose recovered from each stem section are shown in Table 1. Recoveries are reported as grams per gram of depectinated and delignified cell walls. Total carbohydrate extracted decreased with maturity although there was no difference between the upper and lower nodes. The most immature stem tissues (AN) contained a higher proportion of hemicellulose to cellulose as compared to the more mature cell walls (UN and LN). In each case, total hydrolysis of the cell wall residues after extraction indicated that 91%, 92%, and 92% (LN, UN, and AN, respectively) of the neutral sugar content was glucose.

The sequential KOH extraction separated the hemicellulosic material into subfractions differing in chemical composition. The 0.25M KOH fraction contained a large proportion of uronic acids. Subsequent extractions with higher concentrations of KOH solubilized polysaccharides with lower total uronics. With increased maturity (LN) the amount of uronic acid residues increased particularly in the more tightly bound fractions. Neutral sugar composition of subfractions also indicate developmental changes. The arabinose content of the isolated polysaccharides decrease with maturity from 25% in AN stems to less than 5% in LN stems. Polysaccharides isolated from LN stems were composed primarily of xylose (80-85%) in terms of neutral sugar composition. For all maturity levels 4M KOH removes polysaccharides containing 50% glucose. These polysaccharides were not derived from cellulose but probably represent xyloglucans that become tightly associated with cellulose during cell wall synthesis. The hemicellulosic polysaccharides from mature stems appear to be mainly glucuronoxylans with low degrees of substitution. In immature stems the large amount of arabinose indicates more highly substituted arabinoxylans.

It has been proposed that glucuronic acid residues of xylans can form ester linkages with free hydroxyls of lignin. The increased content of glucuronoxylans in maturing stems could enhance this type of interaction resulting in a tighter association with lignin. Decreased substitution by arabinose on xylans would increase the hydrogen bonding to cellulose. Together these two changes in xylans could lead to a tighter cellulose-xylan-lignin matrix which would result in decreased degradation by rumen microorganisms.

Table 1. Hemicellulose extracted from alfalfa stems.

Hemicellulosic polysaccharides extracted from alfalfa stems with a KOH step gradient. Stem fractions include apical nodes AN, upper nodes UN, and lower nodes LN.

M KOH	AN		UN		LN	
	g/g cw	% uronics	g/g cw	% uronics	g/g cw	% uronics
0.25	0.1121	21.91	0.1182	26.92	0.1338	27.47
0.50	0.729	9.82	0.0461	17.51	0.0573	15.83
1.00	0.0863	3.94	0.0494	9.99	0.0474	11.17
4.00	0.1280	3.43	0.0730	6.88	0.0843	9.82

PURIFICATION OF AN ENDO-POLYGALACTURONASE FROM PECTINEX

E.M. SHEA and R.D. HATFIELD

Introduction

Fractionation of the plant cell wall into individual components is usually achieved by chemical means, but the harsh conditions necessary for chemical fractionation can alter the fine structure of the extracted polymers as well as the remaining wall material. Enzymes release wall components selectively by cleaving large polymers at specific sites, but cell wall hydrolases used for structural investigations must first be purified to a single activity by removing any contaminating enzymes. The goal of this work was to isolate and characterize a pectin degrading enzyme from a crude fungal enzyme preparation.

Materials and Methods

A volume of Pectinex 5XL (Novo Enzymes) containing 200 mg of protein was diluted with 20 mM sodium acetate buffer, pH 5.0, and separated into three fractions by ammonium sulfate precipitation. Material precipitated by 50% ammonium sulfate was collected by centrifugation, and the ammonium sulfate concentration in the supernatant was increased to 75%. The insoluble material was removed by centrifugation, and the soluble material was concentrated with an Amicon ultrafiltration device (YM10 filter). The sample was diluted with 20 mM sodium acetate, pH 5.0, and re-concentrated three times to remove excess salt.

The concentrated sample was applied to a DEAE-sepharose column (2.5 cm x 10 cm) and unbound protein eluted with 30 ml of 20 mM Na-acetate, pH 5.0, containing 50 mM NaCl. Bound proteins were eluted with a 400 ml gradient of 350-550 mM NaCl in acetate buffer. Protein concentration in each fraction was monitored by absorbance at 280 nm. All fractions containing protein were assayed for polygalacturonase and cellulase activity with polygalacturonic acid and carboxymethyl cellulose 7MF as substrates.

The fractions containing polygalacturonase were pooled, concentrated, and the buffer was exchanged three times with 10 mM Na-formate, pH 3.5. The sample was applied to an SP-sephadex column (1 cm x 10 cm) and unbound proteins eluted with 30 ml 10 mM Na-formate, pH 3.5. Bound proteins were eluted with a 200 ml gradient of 0-250 mM NaCl in formate buffer. Fractions were assayed for protein, polygalacturonase, and cellulase as above.

The purified polygalacturonase was assayed for other hydrolase activities using the substrates arabinan, arabinogalactan, barley β -glucan, cellulose (powdered Whatman filter paper), carboxymethyl cellulose 4M6F, carboxymethyl cellulose 7MF, galactan, laminarin, locust bean gum, xylan, and xyloglucan. Glycosidase activities were assayed with the *p*-nitrophenyl derivatives of β -galactopyranose, β -glucopyranose, β -glucuronic acid, β -mannopyranose, β -xylopyranose, α -galactopyranose, α -arabinofuranose, and α -fucopyranose.

Results and Discussion

Most of the polygalacturonase activity in Pectinex was soluble in 75% ammonium sulfate. All three fractions contained cellulase activity, but most of the activity was precipitated by 75% ammonium sulfate. Xylanase was precipitated by 50% ammonium sulfate, and only traces of xylanase were in the soluble fraction (Table 1). When separated by DEAE column chromatography, the polygalacturonase eluted with the main protein peak. The cellulase eluted as two peaks on either side of the polygalacturonase peak. When separated on SP-sephadex after DEAE, the cellulase eluted as a separate peak before the polygalacturonase.

The optimum temperature for polygalacturonase activity was 45C, but the enzyme was unstable above 30C for prolonged incubations. Long term incubations were most stable at 25C. The enzyme exhibited maximum activity at pH 4.5 and was most stable between 4.5 and 5.0. Enzyme activity was unaffected when frozen and thawed three times, but activity was reduced 18% when the enzyme was frozen and thawed a fourth time.

The purified polygalacturonase had only traces of activity against barley β -glucan and carboxymethyl cellulose 4M6F when incubated overnight and showed no activity against the other substrates. The amount of these contaminating activities was 500,000-fold less than the polygalacturonase activity. There were no glycosidase activities in the purified enzyme preparation. This three step procedure yields a stable polygalacturonase in large quantities.

Table 1. Total protein and enzyme activities at different steps of polygalacturonase purification from Pectinex 5XL.

Fraction	Total Protein (mg)	Enzyme Specific Activities		
		Xylanase (mg reducing sugar released/min/mg protein)	Cellulase	Polygalacturonase
Crude Pectinex	188.94	0.18	0.01	15.32
50% $(\text{NH}_4)_2\text{SO}_4$ pellet	20.46	0.46	0.02	15.46
75% $(\text{NH}_4)_2\text{SO}_4$ pellet	73.76	0.15	0.06	7.36
75% $(\text{NH}_4)_2\text{SO}_4$ soluble	48.06	0.02	0.01	32.30
DEAE polygalacturonase	20.29	0	0.01	66.54
SP polygalacturonase	8.5	0	0	98.86

EVALUATION OF LIGNIN DEGRADING BASIDIOMYCETES FOR IMPROVEMENT OF LOW QUALITY FORAGES

F.R. VALDEZ, H.G. JUNG, R.A. BLANCHETTE and R.D. HATFIELD

Introduction

Numerous caustic chemicals have been utilized in attempts to improve the digestibility of highly lignified forages and corn residues. Some chemicals such as $\text{NaOH} + \text{H}_2\text{O}_2$ remove large portions of the lignin (~50%) while others like anhydrous NH_3 remove virtually no lignin ($\leq 5\%$). Degree of improvement in digestibility is generally related to the amount of lignin removed by these chemical agents. To date these chemical delignification procedures have been of marginal economic value. They also pose serious questions of safety and pollution control if adopted on a large scale. A possible alternative that may be more environmentally sound and pose less of a safety concern is the use of lignin degrading fungi to improve forage quality. Previous research has resulted in variable response in wheat straw digestibility with fungal pre-treatment. The study described here screened five species of white-rot basidiomycetes, which are selective degraders of lignin from wood, for ability to improve digestibility of different agricultural residues.

Materials and Methods

Phanerochaete chrysosporium (PC), *Sitostroma galactinum* (SG), *Phlebia tremellosus* (PT), *Phellinus pini-2* (PP) and *Pholiota mutabilis* (PM) were used to delignify oat straw and alfalfa stem material. Substrates and fungal cultures were incubated aerobically at 28° C, 90% relative humidity for 30 d. After freeze-drying, the samples were analyzed for cell wall neutral sugars, uronic acids, Klason lignin and esterified phenolics. In vitro dry matter disappearance (IVDMD) was determined by a 48 h fermentation followed by acid-pepsin hydrolysis. Electron microscopy of stem pieces was done using permanganate as a lignin stain and colloidal-gold exo- and endo-cellulase probes.

Results and Discussion

Incubation of oat straw and alfalfa stems with white-rot basidiomycetes resulted in loss of organic matter (Table 1). The greatest organic matter losses were caused by PC and PT in the case of oat straw and both these organisms plus SG for alfalfa stems. There was a net loss of lignin from oat straw due to the action of PC, PT and PP, but no lignin loss was seen from alfalfa. In oat straw the proportions of neutral sugars, uronic acids and Klason lignin did not change due to fungal activity. The net loss of lignin observed was due to a reduction in the total amount of cell wall material in the oat straw. Esterified phenolic acid content of the oat straw cell walls was reduced by PC and PT. The fungi were removing all cell wall components from oat straw non-selectively, except for esterified phenolics, but for alfalfa stems PC, SG, and PT selectively removed neutral sugars and uronic acids leaving cell walls enriched in lignin.

Digestibility of oat straw was increased by PC (Table 1). Inclusion of cell wall content in the statistical model as a covariate indicated that some of the increase in IVDMD was associated with fiber digestion, not just reduced cell wall content. For alfalfa, the fungal incubations generally resulted in a residue of lower IVDMD than the control material. This was expected because of the selective removal of carbohydrate from alfalfa by the fungi.

Staining oat straw stems revealed no differences in lignification due to fungal treatment as expected based on the chemical analysis. Colloidal-gold probes of exo- and endo-cellulases bound to the stem sections, and for oat straw treated with PC there was a significant increase in available binding sites for endo-cellulase. These binding sites were available to ruminal cellulases because rumen fluid fermented stem sections had very few binding sites left for the endo-cellulase probe.

The lignin degrading fungi probably did not remove lignin from alfalfa because in some species of fungi, such as PC, high levels of protein in the media inhibit lignase synthesis. Oat straw digestibility could be improved by at least one fungal species, PC, but the improvement in quality was not sufficient to balance the loss of organic matter. It appears that white-rot basidiomycetes will be of use for improving quality of some crop residues, but the increase in feeding value must be great enough to compensate for the loss of organic matter resulting from fungal activity.

Table 1. Loss of organic matter (OM) and Klason lignin due to fungal incubation, and in vitro dry matter disappearance (IVDMD) of residue.

Forage	Fungal spp	Loss (%)		
		OM	Lignin	IVDMD%
Oat Straw	Control	0	0	34.9
	PC	32.4*	7.7*	44.1*
	SG	3.5*	.4	30.0
	PT	15.2*	3.6*	40.1
	PP	3.5*	1.8*	33.8
	PM	3.1*	.6	32.0
Alfalfa	Control	0	0	55.1
	PC	24.7*	-1.5	37.7*
	SG	20.5*	-2.5*	38.3*
	PT	28.3*	-3.0*	32.7*
	PP	13.0*	-1.8*	44.6*
	PM	10.8*	-1.4	51.3

*Different from appropriate forage source control (P<.05).

VARIATION IN THE EXTRACTABILITY OF ESTERIFIED NON-CORE LIGNIN FROM FORAGES

H.G. JUNG and S.C. SHALITA-JONES

Introduction

Dry matter and fiber digestibility of forages are negatively associated with lignin content of the forage. In recent years interest has been directed toward understanding the role of esterified non-core lignin components of forage cell walls in linking polysaccharides to core lignin and inhibiting digestion. The concentrations of the major non-core lignin components (p-coumaric and ferulic acids) and their ratio to one another in forage material have been shown to be negatively related to fiber digestibility. Virtually all of the data available on these phenolic acids have been collected by

extraction of forage samples with 1 M NaOH after pre-treating the sample with neutral detergent. The objective of the study reported here was to examine the effect of forage species, maturity, pre-treatment and alkali concentration on extractability of esterified p-coumaric and ferulic acids.

Materials and Methods

Whole herbage samples of alfalfa, smooth bromegrass and switchgrass were harvested at vegetative and mature seed stages of growth to provide a broad range of samples. Forage samples were lyophilized and ground through a 1 mm screen in a cyclone mill. Three pre-treatments were tested; an enzymatically derived starch-free alcohol-insoluble residue (AIR), neutral detergent extraction without sodium sulfite (NDF), and NDF subsequently ball-milled to reduce particle size (BM-NDF). Each pre-treated forage sample was then extracted with 0.5, 1.0, 2.0, 4.0 and 6.0 M NaOH for 24 h at 39° C in the dark. After sample clean-up of the filtrate from the extractions, p-coumaric and ferulic acids were quantified by LC.

Results and Discussion

The AIR and NDF pre-treatments resulted in different amounts of organic matter solubilization. As a result, all phenolic acid yields were corrected to the initial organic matter content of the forage samples. Yields also were corrected for recovery of pure p-coumaric and ferulic acid standards from the different alkali concentrations with recoveries consistently being 75 to 80%.

Figure 1 illustrates the overall effects of sample pre-treatment and alkali concentration on extractability of p-coumaric acid from forages. Extractability of ferulic acid gave identical profiles. Both the AIR and NDF preparations resulted in maximal phenolic acid yields when extracted with 1 M NaOH.

Greater concentrations of alkali actually depressed yield, presumably by altering cell wall matrix interactions which interfere with p-coumaric and ferulic acid release. The AIR preparation consistently resulted in greater yields of phenolic acids at all alkali strengths than observed for the NDF preparations. This was expected as esterified phenolics are associated with pectic and hemicellulosic components of the cell wall and the neutral detergent solution solubilizes portions of these components. The greater phenolic acid yields from BM-NDF than NDF also were expected because reduction of particle size generally improves extractability of components. However, the very

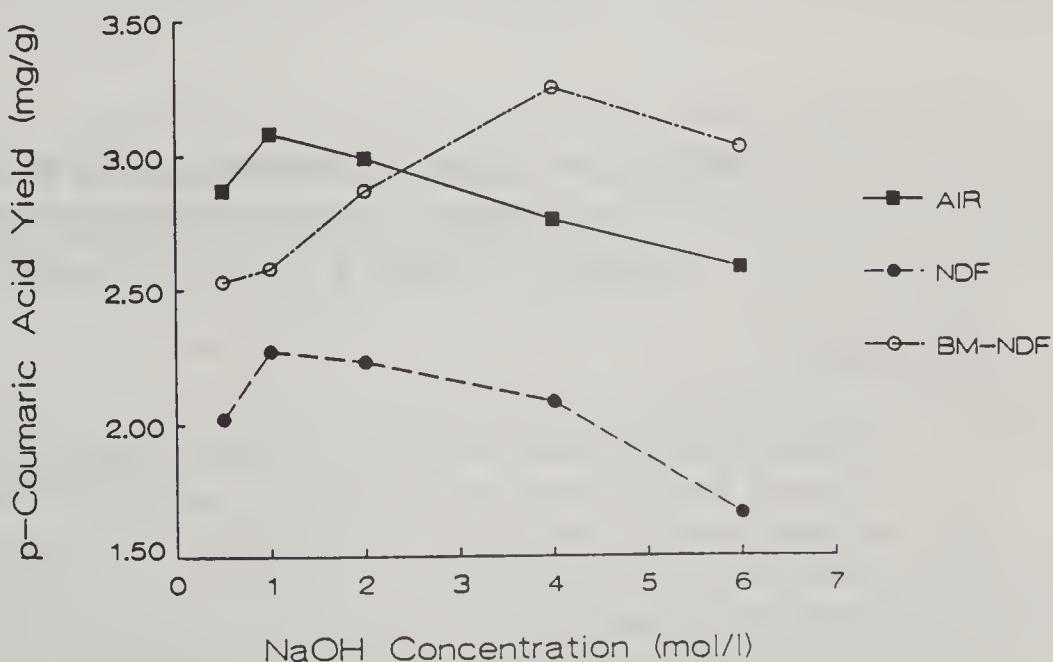


Figure 1. Yield of p-coumaric acid from forages as influenced by sample pre-treatment and alkali concentration. Values are means across all forage samples.

different profile for effect of NaOH concentrations on yield of p-coumaric and ferulic acids for BM-NDF compared to the other preparations suggests chemical changes in the cell wall were induced by ball-milling.

The species of forage and maturity stage of the forage both affected phenolic acid yields. These sample characteristics also significantly interacted with pre-treatment procedure and alkali concentration. Extractability of p-coumaric and ferulic acids from forages was found to be very sample dependent in terms of conditions resulting in maximal yields. Although p-coumaric and ferulic acids responded in a similar fashion to alkali extraction conditions, the molar ratio of these acids varied depending on pre-treatment method.

As a result of this study, several modifications have been made in the standard procedure employed in our laboratory for extraction of esterified phenolic acids from forage cell walls, and these are offered for consideration by others. It appears that use of 1 M NaOH is appropriate for extraction of p-coumaric and ferulic acids from grass species, but 2 M NaOH should be considered for studies of legume species. The use of starch-free AIR preparation is preferable to NDF for quantification of total phenolics in forage cell walls. Until more information is available about the chemical changes induced by ball-milling, reduction of particle size beyond a 1 mm grind is not encouraged. Obviously, removal of esterified p-coumaric and ferulic acids from forage cell wall matrices is a complex process that is subject to variation caused by numerous interacting properties. Interpretation of the role of esterified phenolic acids in ruminal fermentation of forage cell wall polysaccharides will be influenced by the method of analysis. Therefore, careful consideration should be given to the choice of analysis method.

ROLE OF LIGNIN COMPONENTS IN CELL WALL POLYSACCHARIDE FERMENTABILITY

F.R. VALDEZ, H.G. JUNG, R.D. HATFIELD and R.A. BLANCHETTE

Introduction

Total concentration of core lignin in forages is negatively correlated with cell wall digestibility. More recent work has suggested that esterified non-core lignin components and differences in core lignin composition also are responsible for inhibition of fiber fermentability. To assess the relative importance of core lignin concentration vs. composition, or concentration and composition of non-core esterified lignin as determinants of digestibility is very difficult with normal forage material. In general, these lignin components are correlated with each other when compared across forage species or maturity. This study utilized six different methods of delignification to produce forage samples in which the correlated structure among the lignin components was reduced, thereby allowing assessment of the importance of the different lignin fractions as inhibitory factors of fiber digestibility.

Materials and Methods

Alfalfa, smooth bromegrass and corn stalk stem materials were treated with amylase and extracted with alcohol to prepare cell wall preparations. All samples were then delignified by 6 methods: 1 M NaOH, 2 M NaOH-nitrobenzene oxidation, NaOH-H₂O₂, KMnO₄, NaClO₂, and incubation with Phanerochaete chrysosporium. After treatment, residual cell wall material was precipitated from 80% ethanol and freeze-dried. The samples were fermented in vitro for 72 h with a mixed culture of rumen microorganisms to determine digestibility. All samples and fermented residues were analyzed for neutral sugars, uronic acids, Klason lignin, esterified non-core lignin and core lignin composition by nitrobenzene. Cell wall content was defined as the sum of neutral sugars, uronic acids, Klason lignin and esterified non-core lignin.

Results and Discussion

The delignification procedures produced cell wall residues that were different in chemical composition (Table 1). The NaOH and fungal treatments resulted in the loss of cell soluble materials relative to control forages. The composition of the cell walls was altered by delignification. All the chemical procedures removed core lignin, to different extents, while the fungi selectively removed sugar residues. Esterified non-core lignin components were reduced in the cell walls by delignification. There were significant interactions between forage species and delignification procedures which resulted in different ranking of the treatment procedures among the forages. Although the proportion neutral sugars in the cell wall were changed by delignification, the molar ratios of the sugars did not change. Not only did delignification change concentration of core and non-core lignins, but the treatments also caused shifts in molar ratios of esterified p-coumaric and ferulic acids and shifted the core lignin composition towards vanillin.

These changes in lignification resulting from the treatments also were associated with improved digestibility (Table 2). While the fungal treatment did not improve fermentability of neutral sugar components, most of the chemical procedures increased arabinose, xylose and glucose degradability. Uronic acids were already highly digestible in the control forages and did not significantly increase. There were no interactions of forage species and treatment for digestibility. Across all forages, regression analysis indicated that esterified ferulic acid was negatively correlated ($r = -.63$) with arabinose fermentability, whereas core lignin concentration was the primary component related to glucose ($r = -.75$) and xylose ($r = -.80$) digestion. Esterified p-coumaric acid was negatively related to rhamnose fermentability from alfalfa.

The data indicate that fermentability of different polysaccharide components of forage cell walls is influenced by various lignin components. Genetic selection of forages for a particular lignin component may not result in improved digestibility of all cell wall polysaccharides. Because glucose and xylose are the major neutral sugar residues in forage fiber, the data indicate selection against core lignin may represent the most valuable genetic change for improving fiber utilization.

Table 1. Chemical composition of delignified forage cell wall.

Delignification procedure	Cell Wall (% OM)	Percent of Cell Wall			
		Sugars	Uronics	Core Lignin	Non-core Lignin
Control	72.7 ^{ac}	70.4 ^a	7.9 ^{ac}	20.1 ^a	1.6 ^a
NaOH	95.6 ^b	80.9 ^{bd}	7.0 ^a	12.0 ^b	.03 ^b
Nitrobenzene	80.7 ^a	89.7 ^c	4.1 ^b	6.1 ^c	.01 ^b
NaOH-H ₂ O ₂	79.8 ^a	77.1 ^d	10.4 ^c	12.2 ^b	.4 ^c
KMnO ₄	68.3 ^c	82.6 ^b	7.9 ^{ac}	9.1 ^b ^d	.5 ^c
NaClO ₂	68.4 ^c	82.6 ^b	9.7 ^c	7.7 ^{cd}	.1 ^b
Fungi	95.7 ^b	66.8 ^a	6.3 ^{ab}	26.4 ^c	.5 ^c

^{a,b,c,d,e}Means in the same column not sharing a common superscript are different (P<.05.).

Table 2. Fermentability of polysaccharide components from the cell wall of delignified forages.

Delignification Procedure	Digestibility (%)			
	Arabinose	Xylose	Glucose	Uronics
Control	72.2 ^a	43.8 ^a	44.5 ^a	77.8
NaOH	94.7 ^b	87.5 ^{bc}	79.2 ^{bc}	77.0
Nitrobenzene	97.5 ^b	96.8 ^b	85.4 ^b	89.7
NaOH-H ₂ O ₂	97.4 ^b	88.9 ^b	92.5 ^b	90.9
KMnO ₄	81.7 ^a	75.8 ^c	58.2 ^a	74.4
NaClO ₂	87.1 ^b	77.4 ^c	70.0 ^c	74.7
Fungi	63.9 ^a	48.5 ^a	48.0 ^a	73.1

^{a,b,c}Means in the same column not sharing a superscript are different (P<.05).

ALTERATION OF FORAGE FIBER DIGESTIBILITY WITHOUT CHANGING LIGNIFICATION

H.G. JUNG and M.P. RUSSELLE

Introduction

For over 50 years scientists have known that there is a negative correlation between lignin content of forages and their digestibility by ruminants. To date no one has been able to conclusively demonstrate a mechanism by which lignin inhibits forage fiber fermentation. Demonstration of such a cause and effect relationship is difficult because in normally grown forages changes in lignification are associated with many other changes in cell wall structure related to species, plant part and maturity. There have been reports in the literature that light quality, soil nutrient levels, and exogenous

chemicals can inhibit lignin synthesis. The experiment described here attempted to use light quality and soil nutrient levels to alter lignification and/or fiber digestibility of forages.

Materials and Methods

Birdsfoot trefoil and orchardgrass seedlings were grown in sand culture for 4 wk in the greenhouse. After cutting herbage to a 5-cm height the plants were moved to growth chambers fitted with either full-spectrum fluorescent-incandescent (FI) lamps or low-pressure sodium (LPS) lamps producing primarily yellow light. Within each lighting regime the forages were grown with total nutrients, high NH_4^+ :low NO_3^- , low NH_4^+ :high NO_3^- or low sulfur nutrient solutions. After 4 wk of regrowth, plants were harvested at ground level. All samples were lyophilized. Birdsfoot trefoil plants were separated into leaf and stem tissue. The orchardgrass did not produce stem tissue. In vitro ruminal fermentations were done for 48 h. All samples and in vitro residues were analyzed for detergent fiber components.

Results and Discussion

The birdsfoot trefoil plants on the high NH_4^+ :low NH_3 nutrient treatment did not exhibit normal growth characteristics and the data have been discarded. Total dry matter yield of birdsfoot trefoil, but not orchardgrass, was depressed 24% by the LPS lighting. Light source did not influence leaf to stem ratio in birdsfoot trefoil, but the low NH_4^+ :high NO_3^- and low sulfur nutrient treatments depressed leaf to stem ratio somewhat.

Detergent fiber component makeup of the forages changed slightly in response to the treatments. In birdsfoot trefoil leaves the low NH_4^+ :high NO_3^- treatment reduced total NDF concentration and increased the cellulose content of the NDF under both FI and LPS lighting. The low NH_4^+ :high NO_3^- treatment also reduced NDF content of birdsfoot trefoil stem material for both lighting treatments. The NDF content of orchardgrass was increased by the LPS treatment and the high NH_4^+ :low NO_3^- nutrient regime. Lignin content of the fiber was not affected by any treatment for either forage.

Digestibility of fiber fractions was altered by the treatments (Table 1). Birdsfoot trefoil leaves had greater NDF digestibility on the low NH_4^+ :high NO_3^- treatment than the other nutrient regimes. Hemicellulose digestibility of leaf tissue was greater for LPS grown plants than those under FI lights on the total nutrient and low sulfur treatments, but not the low NH_4^+ :high NO_3^- regime. Across all nutrient treatments, cellulose digestibility of birdsfoot trefoil leaves was greater for the LPS light source. Stem tissue from birdsfoot trefoil consistently had greater NDF, hemicellulose and cellulose digestibilities on the LPS lights, across all nutrients regimes. In contrast to the increases in digestibility seen for birdsfoot trefoil, statistical analysis indicated an overall depression in digestibility of all detergent fiber components for LPS grown orchardgrass compared to the FI lighting. The reductions were small, 1.8 to 3.0%.

The inability to detect changes in lignification due to the environmental treatments may relate to the immature state of the forage and, therefore, low concentrations of lignin present. The data do suggest, however, that simple measures of lignin concentrations are not always related to fiber digestibility. Greater attention should be placed on evaluating the role of lignin structure and other elements of the cell wall matrix as mechanisms for limiting fiber degradability.

Table 1. Digestibility of detergent fiber fractions during 48 h in vitro ruminal fermentations.

Species	Plant	Fertility	Digestibility (%)					
			NDF		HC		CEL	
			Trt	FI	LPS	FI	LPS	FI
Birdsfoot trefoil	leaf	Total	57.2	62.7	54.9	72.6	89.8	93.3
		NO ₃	65.8	68.2	68.4	69.4	92.7	96.4
		S	58.2	63.4	57.1	70.8	88.6	93.6
	stem	Total	43.0	49.8	34.7	48.9	55.6	63.6
		NO ₃	42.5	49.7	35.2	47.1	56.0	62.2
		S	42.7	49.7	38.9	50.7	54.9	63.1
Orchard- grass	leaf	Total	63.0	65.2	64.2	63.9	65.8	68.4
		NH ₄ ⁺	67.0	67.9	68.6	69.1	70.5	70.7
		NH ₃	70.4	70.3	70.1	69.6	76.1	73.9
		S	64.2	56.4	65.7	57.7	67.3	57.5

QUICK-DRYING FORAGE MATS

R.G. KOEGEL, K.J. SHINNERS and R.J. STRAUB

Introduction

Mats made from alfalfa at the time of mowing and placed on stubble have been shown in earlier research to dry to a moisture content suitable for baling in 2 1/2 to 6 hours under favorable conditions. Furthermore, alfalfa so harvested proved to have more rapid and extensive NDF digestion than conventionally harvested material.

Efforts during the past year have been concentrated in two areas: (1) technology for picking up and handling the field-cured mats and (2) improving the strength and uniformity of the mats.

Materials and Methods

Since the normal tine-and-drum pick-up used on most balers and forage harvesters is not well-suited to picking up forage mats, an alternative pick-up was designed, built and evaluated. This unit consisted of a wide belt the upper surface of which formed an inclined plane. Plastic tines were attached to the belt to make it more aggressive. The belt was driven by the carrier wheels of the unit in such a way the its horizontal component of velocity was equal and opposite to ground speed. This resulted in the mats generally being raised intact to the harvesting device.

The belt pick-up was adapted to two standard forage harvesting machines for making silage or dry hay respectively: (1) A flail chopper from which the flail had been removed, so that the mats were fed directly by the pick-up unit into the cross auger and thence into the chopper-blower unit for delivery to a traile wagon. (2) A conventional rectangular hay baler from which the original pick-up had been removed.

A device for measuring the dynamic strength of mats as they came from the press was also designed and built. The strength of mats was measured in terms of the maximum unsupported distance which they would span without tearing.

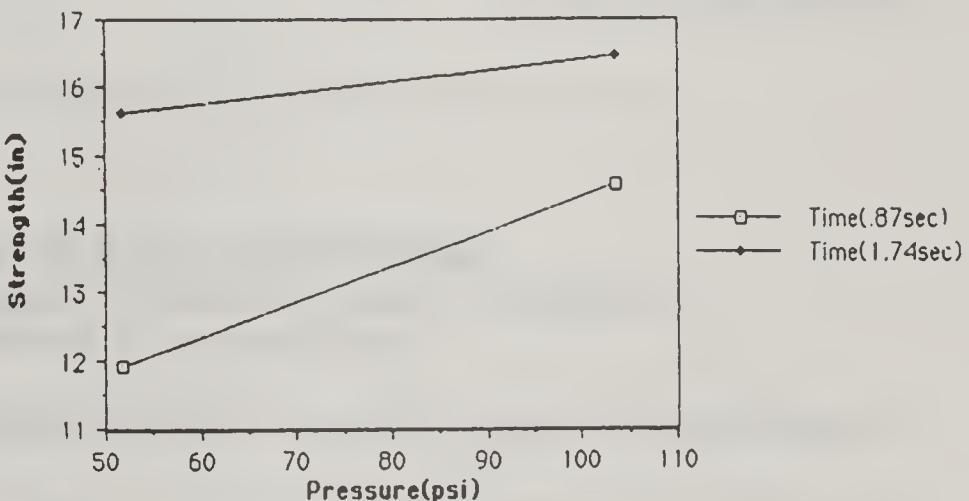


Figure 1. Effect of pressure of formation.

Results and Discussion

The belt pick-up functioned as intended. It lifted the mats cleanly from the stubble and delivered them generally intact to the machine of choice.

The modified flail chopper broke up the mats effectively in the cross auger, chopper and blower and delivered them to the traile wagon. Alfalfa thus harvested was ensiled at approximately 50% moisture content in a pilot silo of 2000# capacity. A control silo of conventionally harvested material was also filled. Feeding trials with sheep comparing these two material have been carried out, but results are not yet available.

Rectangular bales were successfully made from the mat material. Because of the compliancy of the shredded forage, bales tended to be less rigid for a given density. It was therefore necessary to add more resistance to the bale chamber in the form of wedges. Observations indicated that bale chamber losses were comparable to those from conventional baling operations. However, accurate loss measurements have not yet been made.

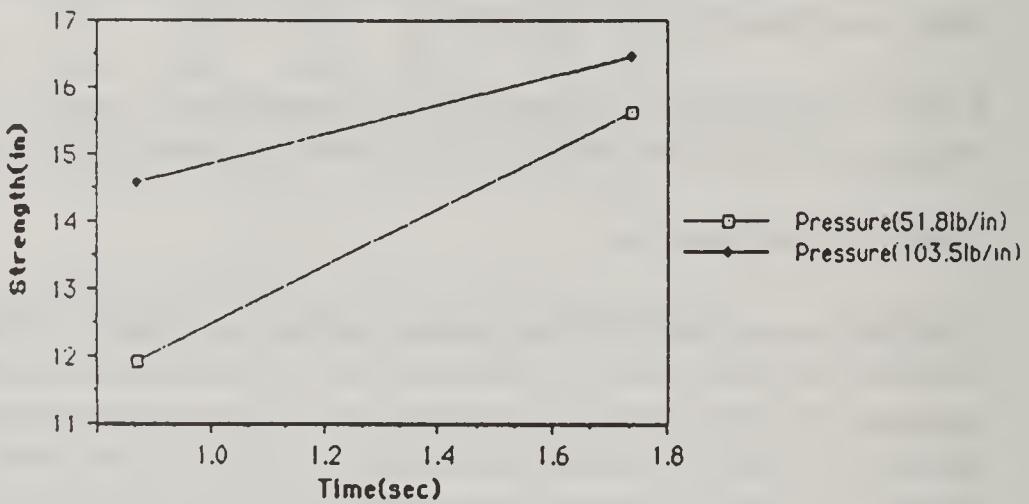


Figure 2. Effect of time of formation.

A series of trials of dynamic mat strength confirmed that both pressure and pressing time contribute to mat strength (Fig 1 and 2).

INCREASING FORAGE VALUE

U.S. SIROHI, B.J. HONG, C. KELENY, R.G. KOEGEL, G.A. BRODERICK,
R.J. STRAUB and K.J. SHINNERS

I. Mechanical and Chemical Treatments to Improve Alfalfa Stem Digestibility

Introduction

Intensive conditioning or maceration has been carried out on forages to increase their drying rates. Some work has indicated that such mechanical processing may also improve the digestibility of the forage. Several chemical treatments have also been applied to plant material to increase the rate and

extent of hydrolysis in the production of ethanol. A maceration treatment and several chemical treatments alone or in combination were evaluated for their effect on rate and extent of in vitro NDF digestibility.

Materials and Methods

Alfalfa stems were hand separated from leaves. Maceration took place in a prototype unit consisting of 6 planetary rollers of 50mm diameters arranged around a cylinder of 200mm diameter. All surfaces were knurled and the rollers were driven at a peripheral speed approximately one-half that of the cylinder. The ammoniation treatment consisted of "soaking" the alfalfa stems in ammonia gas at 800 kPa pressure for 30 minutes while the "explosion" treatment consisted of soaking the alfalfa stems in nitrogen gas at 3450 kPa for 30 minutes and then suddenly venting the chamber to the atmosphere. The treated alfalfa was evaluated by determining NDF digestion using a rumen in vitro digestion procedure.

Results and Discussion

Results of the single treatments and a triple treatment combining maceration, ammoniation, and explosion are compared to the control in Fig 1. All single treatments increased the digestion relative to the control with maceration being the most effective single treatment. The triple treatment combining maceration, ammoniation, and explosion was, however, more effective than maceration alone. At 12h and 24h maceration resulted in a 39% increase in digestibility over the control while the triple treatment resulted in approximately a 50% increase over the control.

Conclusions

In addition to increasing the drying rate of alfalfa, the maceration process appears to have the potential for increasing the digestibility. In vivo studies are needed to estimate this increase.

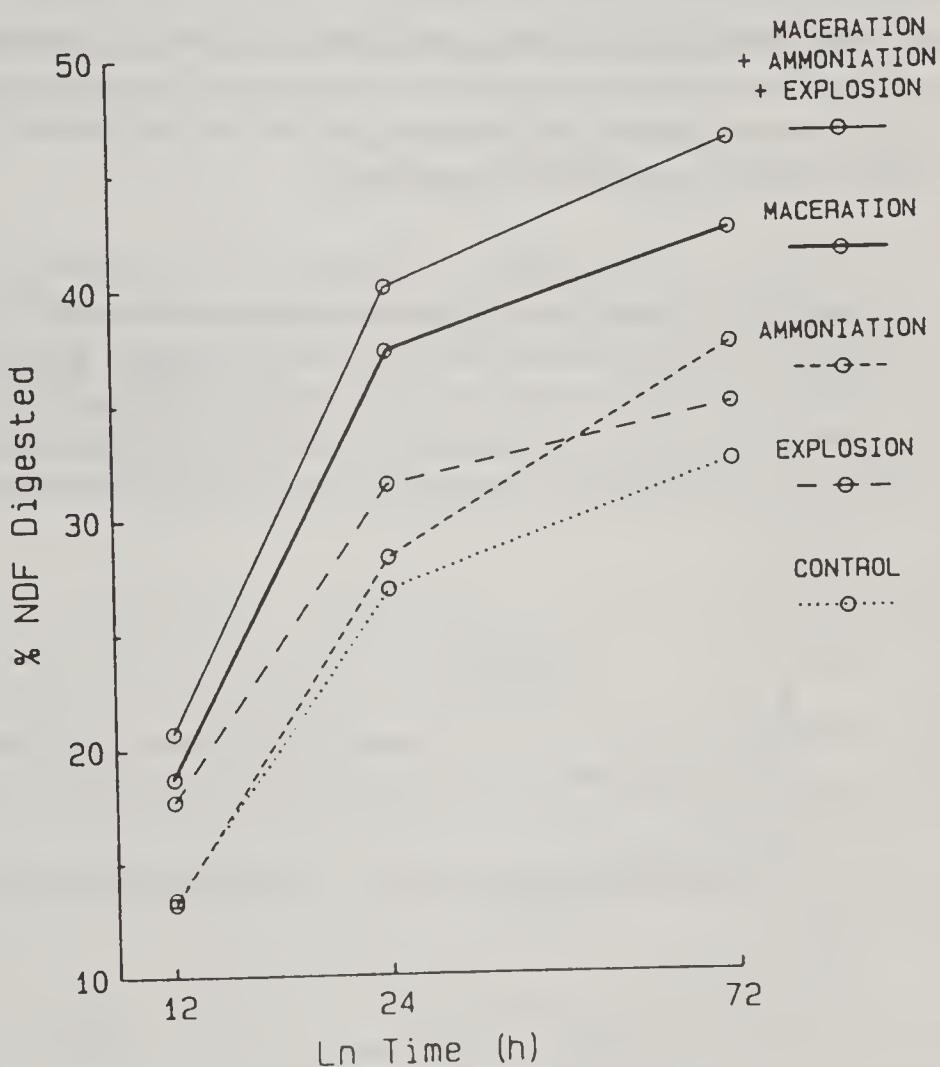


Figure 1. Single and triple treatments compared with control.

II. Densification of Forages to Decrease Shipping Costs

Introduction

Because of its low density, approximately one-fourth that of grain, baled forage can generally not be shipped more than a few hundred miles profitably. This greatly limits the shipping of forages from surplus to deficit areas or from areas where cost of production and/or quality is favorable to less favored areas. Tripling the density of forages could greatly help to overcome these limitations.

Materials and Methods

Baled alfalfa at densities of approximately 130 kg/m^3 was further compressed at varidus: (1) pressures up to 20.7 MPa, (2) temperatures, and (3) holding times. The densities of the resulting materials were monitored periodically after release of pressure.

Results and Discussion

The order of importance of the variables was (1) pressure, (2) temperature, and (3) hold time. An increase in each variable resulted in an increase in final equilibrium density after pressure release. Fig 2 shows the effect of two pressure levels on relaxed density. Effects of temperature and holding time are similar, but smaller in magnitude.

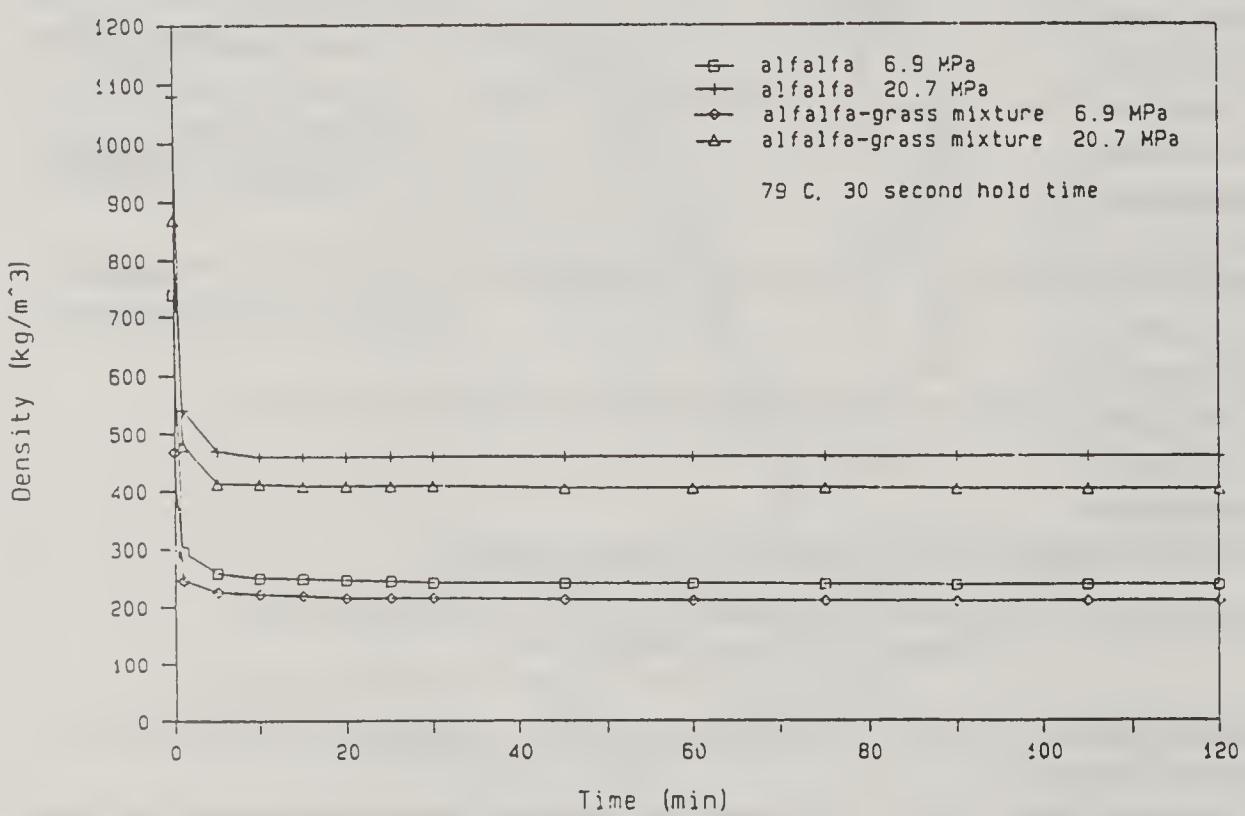


Figure 2.
The effect of
pressure on alfalfa
and alfalfa-grass
mixtures.

Conclusion

With proper compression equipment it should be possible to triple the density of baled forages prior to shipment which should approximately reduce the per ton transport cost to one-third of that for conventional bales.

FORAGE FIBER RECOVERY FROM DAIRY WASTE

J.Y. KIM, R.G. KOEGEL, W.P. DELZER and R.J. STRAUB

Introduction

Undigested forage fiber can be recovered from dairy manure and become a value-added product when used as bedding, for feeding, as a soil amendment, or as a biomass fuel. In addition, separation of the fibrous fraction of dairy waste facilitates the storage and handling of the remaining liquid fraction. Separation devices, available to date, have had problems which include high initial and operating costs, low throughput, and lack of reliability.

Materials and Methods

Performance characteristics of these types of separation presses were evaluated: (1) a perforated roll press with eight rolls, (2) a 16 inch diameter screw press fed from an inclined separator screen, and (3) a reciprocating press which was under development. Characteristics of particular interest were: (1) influent throughput, (2) fiber recovery ratio, and (3) fiber solids content as functions of press configuration and influent solids content.

Results and Discussion

Some typical results for the multiple perforated roll press are given in Figs 1 and 2 and for the reciprocating press in Figs 3 and 4. While the screw press produced a fiber output of high solids content (>40%), its initial cost and power requirement were high and its throughput low relative to the other presses. This appeared to eliminate it from practical consideration. While the performance of the roll press and the reciprocating press were somewhat comparable, the smaller size and greater simplicity of the reciprocating press would appear to give it advantages in initial cost and reliability.

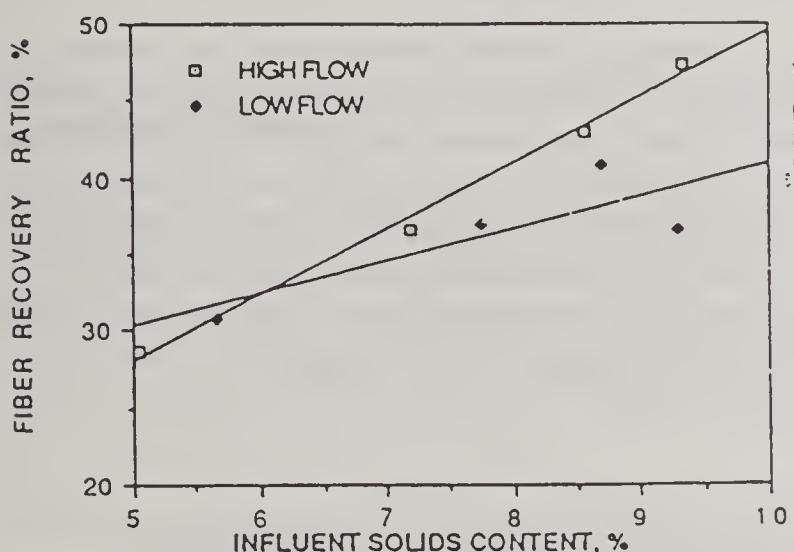


Figure 1. Fiber recovery ratio vs influent solids content.

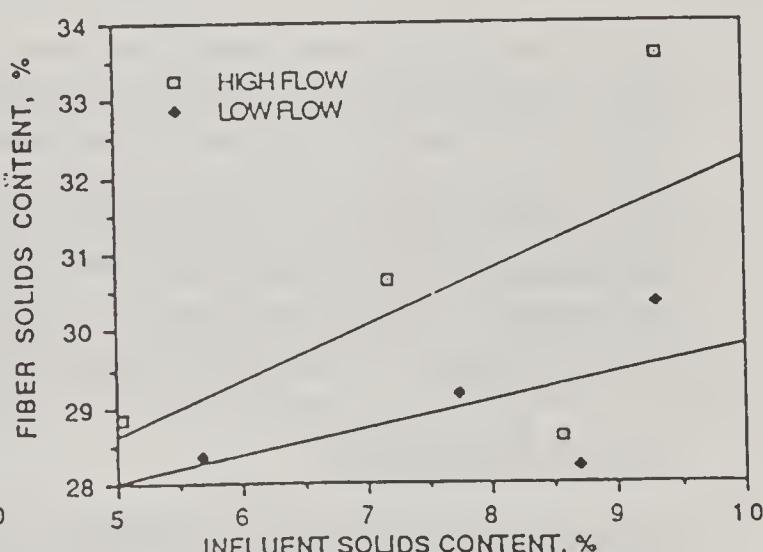


Figure 2. Fiber solids content vs influent solids content.

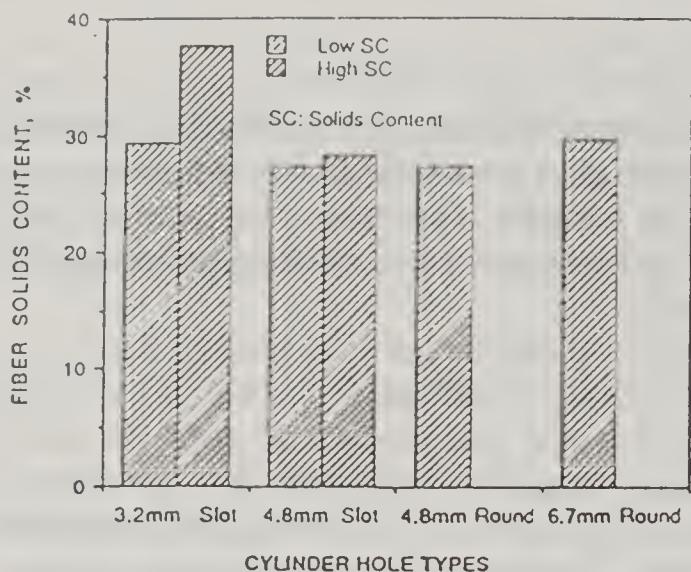


Figure 3. Fiber solids content vs different cylinder hole types.

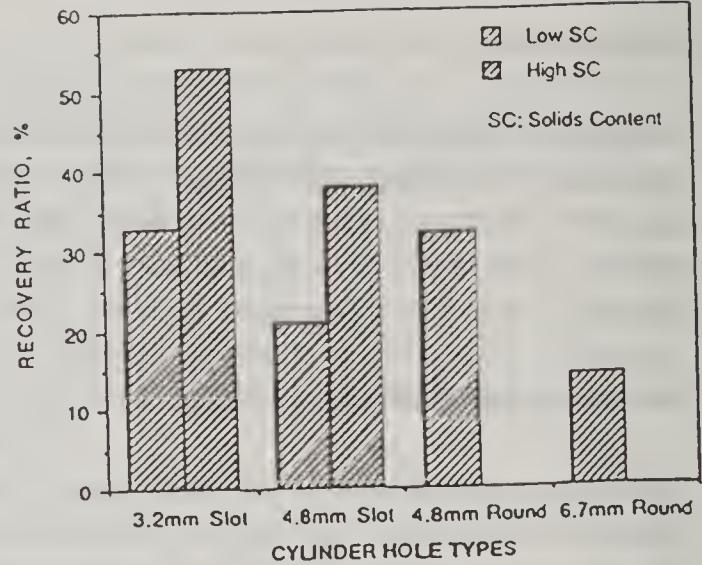


Figure 4. Ratio of dried solids weight in fiber to that in influent vs different cylinder hole types.

VALUE OF LOSSES IN ALFALFA HARVEST AND STORAGE ON A DAIRY FARM

D.R. BUCKMASTER, C.A. ROTZ, and J.R. BLACK

Introduction

Large losses normally occur during the harvest and storage of alfalfa hay and silage on the dairy farm. The effect these losses have on forage quality and ultimately animal performance is not well understood. In order to prioritize research needs in alfalfa harvest and storage, this type of information is needed. Research dollars should be invested in those parts of the system which promise to provide the greatest return, and the greatest return will likely be obtained by improving those parts which cause the greatest loss of value. The objective of this work was to determine the impact of each definable loss on animal performance, feed value and ultimately farm profitability. Sources of loss were compared to determine those which caused the greatest loss in forage value.

Materials and Methods

The value of losses was determined using a simulation model of the dairy forage system (DAFOSYM). DAFOSYM is a comprehensive model which simulates growth, harvest, storage and feed utilization of alfalfa and corn on representative dairy farms. An economic return for the farm is determined by comparing production and feed costs to the income from milk produced. Long-term comparisons are made by simulating the processes over 26 years of weather conditions.

All major losses in alfalfa harvest, storage, and utilization were modeled in DAFOSYM for typical dairy farms in the northern U.S. These losses included: respiration and rain damage during field curing, mechanical losses from mowing, raking, baling and chopping, and storage losses for dry and ensiled forage. The value of each loss was determined by independently eliminating the loss in the model and measuring the impact the change had on farm income. The change in farm income was divided by the quantity of alfalfa processed to give the value of the loss per unit of forage produced.

Table 1. Average losses in alfalfa harvest and storage on a typical dairy farm and their value when forage is fed to moderate (low) and high (high) producing dairy cows. Values are based upon a 26-year simulation of a 100-cow dairy farm using good management practices where first and fourth cuttings are harvested as silage and second and third cuttings are harvested as hay.

Source of loss	DM	Change in		Loss in value	
	Loss (%)	CP (%)	NDF (%)	low - \$/T DM -	high
Respiration	2.1	0.5	0.9	1.32	6.24
Rain	3.1	-0.2	1.1	2.94	7.08
Machinery	10.7	-0.4	0.8	8.52	13.62
Mower-conditioner	2.1	-0.1	0.2	1.66	2.46
Rake	7.8	-0.3	0.6	7.01	10.00
Baler	5.0	-0.3	0.5	3.79	5.93
Chopper	2.6	-0.2	0.4	3.00	4.82
Inside storage of hay	2.8	0.3	1.2	9.03	19.15
Outside storage of hay*	14.0	0.0	7.0	17.39	66.30
Silo, stave**	8.7	2.1	3.7	6.42	28.93
sealed	8.1	2.0	3.7	6.38	28.51
Bunker	9.1	2.0	3.7	6.85	30.58

* Large round bales

** Average moisture content of alfalfa entering the silo was 60, 55 and 65% for stave, sealed and bunker silos, respectively.

Results and Discussion

The impact of each loss on the quantity and quality of alfalfa produced was determined for a typical dairy farm of 100 cows and 50 ha of alfalfa (table 1). Values were determined for two types of herds: 1) a herd with a moderate production level, and 2) a herd with a very high production potential limited only by the quality of the forage fed. Production potential had a great impact on the value of losses, but the ranking of how loss values compared to one another was not very different between the two production levels.

Losses were greatest during storage, particularly for ensiled forage. Rain losses can be very high on any particular crop, but when averaged over all alfalfa produced, the loss in value due to rain damage was not that high. Machine losses were moderately high in value with the greatest loss occurring during raking.

Conclusions

Future research should be directed toward reducing storage losses because these losses have 2 or 3 times the value of most harvest losses. Research on harvest systems should also be continued toward reducing the need for mechanical handling of the crop and/or speeding the field curing process. Harvest system changes must not increase production costs more than the value of the forage saved which would normally be less than \$7 per ton of forage treated.

ECONOMICS OF MACERATION AND MAT DRYING OF ALFALFA ON DAIRY FARMS

C.A. ROTZ, R.G. KOEGEL, K.J. SHINNERS and R.J. STRAUB

Introduction

A new mat process for harvesting forage is under development which provides rapid field curing of hay. A mat making machine is used to mow, macerate and press the forage into a mat. The mat is laid back on the field where it dries up to 3 times faster than conventionally-mowed hay. The dry mat is picked up with either a modified large round baler, a modified forage chopper or a mat harvester. A mat harvester is a machine designed to pick up the mat, break it apart, and transfer the forage onto a trailing wagon. The same machine could be used to harvest mats as either hay or silage.

Small field machines have been built and used for making and harvesting mats to demonstrate the feasibility of the new process. The unanswered question is the economic viability of the process. The increase in the quantity and quality of the forage produced by the process must improve farm production enough to more than offset any increased costs of the process.

To compare the benefits and costs of this new process to conventional harvest methods, a modeling study was needed. A simulation model of the dairy forage system (DAFOSYM) was previously developed for analyzing the long-term benefits of new technology in forage harvesting. A modeling study was undertaken to 1) develop models of drying rate and loss for alfalfa field-cured in mats, 2) incorporate these models and other parameters required to describe the mat process into DAFOSYM, and 3) use DAFOSYM to analyze the economics of using the process on representative dairy farms of the northern U.S. for alfalfa hay and silage production.

Materials and Methods

Drying rate and loss models were developed from experimental data collected on field-cured mats. The drying rate of matted alfalfa was a function of the drying rate of a conventional swath and the area density of the mat. Machine losses were similar or slightly lower than those used for conventional equipment. Loss due to rain damage, although infrequently occurring was about 6 times greater than that from conventional swaths.

Machine parameters were estimated based upon measurements from experimental mat processing equipment, and a comparison of this equipment to other conventional farm equipment. Machine parameters included: initial cost, power requirement, field speed, field efficiency, operating width, and mass. These parameters and models were incorporated into DAFOSYM to simulate the new system.

System comparisons were made using 26-year simulations for several representative dairy farms at East Lansing, Michigan. Comparisons were for both moderate and high producing dairy herds with farms of 60, 100 and 150 milking animals. The new process was evaluated in all hay, all silage and mixed hay and silage systems. The mat process was modeled both with and without an assumption that maceration increased the digestibility and intake of alfalfa. With the assumption, digestibility of fiber was increased 13% and intake was increased 6%.

Results and Discussion

Maceration and mat drying was economical when used in hay production on a dairy farm. The reduction in field losses decreased feed costs enough to more than offset the increased costs of production. When maceration was assumed to improve the digestibility and intake of forages, \$5.00 was returned to the farmer for each dollar spent on increased machinery costs.

Implementation of maceration and mat drying in silage production had limited benefit. When maceration was used on a farm with a high producing herd, and maceration was assumed to improve the digestibility and intake of forages, the new process just paid for itself. With a lower producing herd where milk production was not limited by forage quality, the benefit from maceration of alfalfa for silage did not justify the cost.

The economic benefit of the mat process was increased dramatically on farms which produced both hay and silage when a mat harvesting machine was used to harvest both forms of forage. The machinery investment was reduced which provided a return of up to \$45 for each dollar spent on increased machinery costs.

The economic benefit of the mat system was very sensitive to the production potential of the herd, the increase in digestibility obtained through maceration, the initial cost and power requirement of the mat making machine, and the drying rate of the forage mat. The loss of forage due to machine treatments or rain damage had a small to moderate impact on the economic benefit of the new harvest system.

Conclusions

The economic benefit of maceration and mat harvesting of alfalfa is dependent upon the way in which the dried mat is harvested from the field. When a modified large round baler or forage chopper are used to pick up the mat, the process is very economical in hay production but not in silage production. On farms which produce both hay and silage, the mat process can be very economical by adapting the entire harvest and storage system to a mat harvester which harvests both hay and silage.

NUTRITIVE VALUE AND ENSILING CHARACTERISTICS OF MAIZE AS INFLUENCED BY AGRONOMIC FACTORS

J.R. RUSSELL, N.A. IRBECK, A.R. HALLAUER and D.R. BUXTON

Introduction

Little information is available for selecting maize hybrids for silage and the optimal plant density and harvest date for high-quality silage. Often farmers are advised to use those hybrids and practices that result in greatest grain production because of the high nutritive value of grain. The quantity of nonstructural carbohydrates in maize stover, however, is inversely related to the proportion of grain in the herbage and can compensate for low grain yield. Also, farmers have at times raised questions about the value of silage production if seed were saved from hybrids (F_1 generation) and used for the next year's crop (F_2 generation). A study was conducted in Iowa to investigate these relationships.

Materials and Methods

A 2-yr experiment was conducted to investigate the effect of genotype, generation of hybrid (F_1 vs F_2), plant density and harvest date on the composition and ensiling characteristics of total herbage maize. In Yr 1, three maize hybrids (A632 x LH38, early-season; B73 x Mo17, mid-season; B73 x Pa91, full season) were grown in 3 replicated plots at densities of 4.9, 6.9 and 8.9 plants m^{-2} (20,000, 28,000, and 36,000 plants $acre^{-1}$) and harvested at 0, 14, 28 d after physiological maturity. In Yr 2, the F_1 and F_2 generations of the hybrids used in Yr 1 were grown and harvested at physiological maturity. Samples were taken for both fresh-harvested forage and silage prepared in polyethylene bags.

Results and Discussion

The A632 x LH38 hybrid had a lower grain yield, grain-to-stover ratio ($P<0.01$) and concentrations of dry matter (DM); and in vitro digestible DM (IVDDM) in the forages at physiological maturity than other hybrids. Silage from A632 x LH38 maize had lower concentrations of lactic acid and acetic acid than the other hybrids.

Density did not affect grain yields or grain-to-stover ratios. Maize grown at 6.9 plants m^{-2} had higher concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) than did maize grown at either higher or lower densities (Table 1).

In Yr 1, later harvest resulted in higher grain-to-stover ratios, higher concentrations of DM, ADL, and CP in forages, lower concentrations of ADF and TNC in forages and lower concentrations of lactic acid and acetic acid in the silages.

In Yr 1, F_2 generation maize had lower grain yields ($P<0.01$) than F_1 generation maize, particularly for the B73 x Pa91. Forages from F_2 generation maize also had lower grain-to-stover ratios, lower

concentrations of IVDDM and TNC and higher concentrations of NDF, ADF, ADL, and CP. Silages from F_2 generation maize had lower concentrations of acetic acid. Interactions between plant density and generation for the concentrations of IVDDM and ADF showed less density tolerance in the F_2 than in the F_1 generation of hybrid.

The results imply that grain-to-stover ratios of maize ranging from 0.66 to 1.26 do not greatly influence the digestibility of fresh-harvested or ensiled maize herbage. Therefore, management practices that increase yield such as using full season hybrids or harvesting at physiological maturity will not greatly decrease maize silage digestibility even though they may alter the grain-to-stover ratio. Similarly, utilizing the F_2 generation of the maize hybrids decreased the grain-to-stover ratio of the forage while only having small effects on its digestibility.

Table 1. Effect of corn hybrid and plant density on the composition of fresh-harvested and ensiled maize forage¹.

Hybrid	Plant density (plants m^{-2})	DM (g kg^{-1})	Proportion of DM (g kg^{-1})					
			IVDDM	NDF	ADF	ADL	TNC	CP
A632xLH38 (early season)	4.9	467	750	455	236	36	340	69
	6.9	457	748	455	241	37	367	70
	8.9	430	761	457	234	35	346	74
B73xMo17 (mid season)	4.9	381	750	459	237	36	358	66
	6.9	388	721	503	274	45	314	64
	8.9	373	739	466	245	38	332	61
B73xPa91 (full season)	4.9	407	736	431	227	36	356	71
	6.9	377	735	452	245	8	376	73
	8.9	400	720	465	252	38	331	66
SEM		14.5	9.7	9.8	6.3	1.9	14.5	2.1
Significance								
Hybrid (h)		<0.01	0.04	0.01	0.03	0.09	0.22	<0.01
Plant density (d)		0.35	0.40	0.04	<0.01	0.04	0.37	0.45
h x d		0.40	0.32	0.09	0.04	0.20	0.12	0.12

¹Values are means of 3 replications of the F_1 generation of fresh-harvested and ensiled maize harvested at physiological maturity.

EFFECT OF INOCULATION LEVEL ON ALFALFA SILAGE QUALITY

R.E. MUCK and P.L. O'CONNOR

Introduction

Bacterial inoculants supplying lactic acid bacteria (LAB) are common additives for ensuring a rapid fermentation of alfalfa in the silo. When inoculants are effective, pH declines more rapidly, final pH is lower, lactic acid represents a larger fraction of the fermentation products and more true protein is preserved. However, studies have not always shown inoculants to be effective.

One reason for inoculant failure is the number of natural or epiphytic LAB on the crop at ensiling. If the epiphytic LAB level is high, then the inoculant LAB may not dominate the fermentation. Research at the Dairy Forage Research Center has shown that animal production responses to silage inoculation occur only when the inoculant LAB is applied at a rate 10 times that of the epiphytic LAB. Silage quality in those trials, as indicated by final pH and lactic to acetic acid ratio, was improved in all but two cases. Both times the epiphytic LAB population was approximately equal to that supplied by the inoculant. These results suggested that silage quality improvements from inoculation were more easily obtained than animal production responses.

Thus, the objective of this study was to determine the relative level with respect to the epiphytic LAB population at which an inoculant would fail to affect silage quality.

Materials and Methods

Alfalfa was ensiled in mini-silos in each of four harvests (June 10, July 13, August 12 and October 7). The alfalfa was harvested normally and refrigerated at 4°C overnight to guarantee sufficiently large numbers of epiphytic LAB at ensiling. The following morning the alfalfa was ensiled with an inoculant applied at various dilutions to provide 0 (Control), 10^3 (Inoculum 1), 10^4 (Inoculum 2), 10^5 (Inoculum 3), and 10^6 (Inoculum 4) colony forming units (CFU) of LAB/g alfalfa. The inoculant contained Streptococcus faecium, Lactobacillus plantarum and Pediococcus species and had been shown in previous trials to be an effective inoculant. Fifteen silos of each treatment were made, placed in a water bath and incubated at 30°C.

At 12 h, 1, 2, 4 and 40 d, three silos of each treatment were removed from the water bath, frozen immediately and stored at -18°C until time for analysis. The initial alfalfa samples and the silages were analyzed for pH, moisture content, total Kjeldahl nitrogen, soluble non-protein nitrogen (NPN), free amino acids, ammonia (NH3) and fermentation products (lactate, acetate, succinate, formate, propionate, 2,3-butanediol, ethanol, butyrate). The initial alfalfa samples were also analyzed for LAB, buffering capacity, total non-structural carbohydrates and reducing sugars.

Results and Discussion

Table 1 indicates that a wide range of dry matter levels, sugar contents and epiphytic LAB numbers were tested in the four trials. Inspite of this variation, the results of the trials were surprisingly consistent.

Inoculation failed to provide improvements in all cases and for all silage characteristics measured at 40 days when the inoculum LAB level was less than 1% of the epiphytic LAB population. When the inoculum level was close or equal to the epiphytic LAB population, lactic acid concentration was consistently increased with respect to the control silage. Final acetic acid content and pH were not always lowered under such conditions; however, non-significant effects were associated with sugar-limited silages.

The time-course changes were more consistent than the effects on final silage quality. The inoculant began to affect fermentation at an application level approximately 1% of the epiphytic population (Figure 1). At 10% of the epiphytic LAB population, the inoculant always lowered silage pH and increased lactic acid concentration more quickly than the control.

The practical implications of this work are that silage quality effects will be found over a wider range of conditions than effects on animal performance. An inoculant supplying 10^5 CFU/g alfalfa

should show a silage quality benefit as long as the epiphytic LAB level is less than 10^6 CFU/g. Under Wisconsin conditions, epiphytic levels appear to be less than 10^6 approximately 90% of the time. The same inoculant will provide an animal response when epiphytic levels are below 10^4 CFU/g alfalfa, a condition which occurs only 50% of the time in Wisconsin. Consequently, there will be a substantial percentage of cases where an inoculant will give the farmer the impression that it worked (e.g., less fermented smell), but little or no economic benefit from animal performance will be realized.

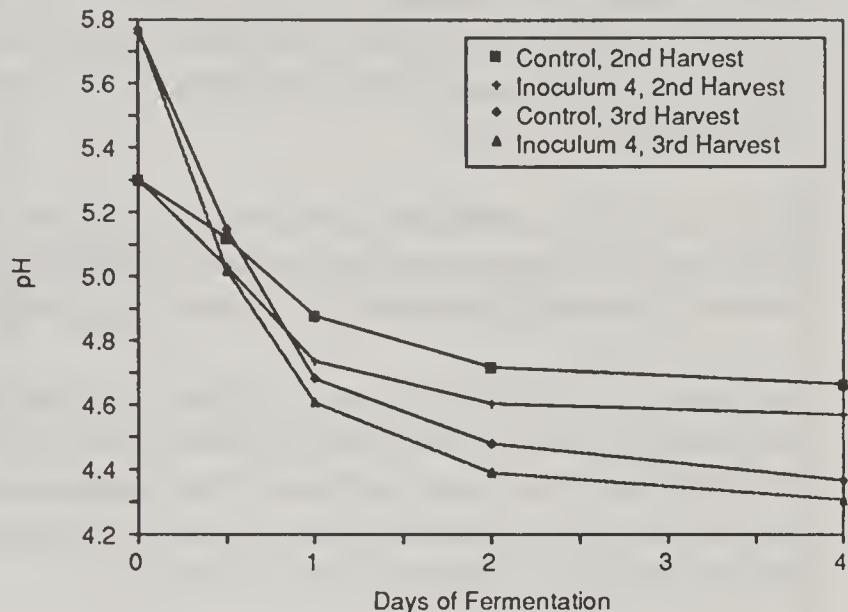


Figure 1. Second and third harvest pH time courses.

Table 1. Characteristics of alfalfa ensiled in each harvest.

	Harvest			
	1	2	3	4
Dry Matter, %	49.4	36.7	39.0	18.1
TNC, % DM	4.4	3.7	5.7	7.3
Sugar, % DM	2.8	1.6	3.5	4.9
Buffering Capacity, meq/kg DM	458	473	391	556
Crude Protein, % DM	17.8	21.2	18.8	22.1
Epiphytic LAB, Log(CFU/g)	5.07	9.39	8.23	6.56
Inoculum 4, Log(CFU/g)	6.60	6.93	6.63	6.61

DRY MATTER LEVEL EFFECTS ON SILAGE FERMENTATION PRODUCTS AND STARCH HYDROLYSIS

R.E. MUCK

Introduction

The dry matter (DM) content at which alfalfa is harvested for ensiling in the United States varies widely depending on the farmer, the type of storage and environmental conditions. Because of this variation, it is important to know any effects of DM content on fermentation that would alter the best management of the crop.

Effects of ensiling alfalfa and other forages at the extremes are well known. Ensiling unwilted alfalfa leads to effluent losses and possibly clostridial silages. Ensiling high DM (>60%) alfalfa increases harvesting losses and the chances for heat-damaged silages.

For grasses, increasing DM content typically has reduced the amount of fermentation resulting in increased final pH and water soluble carbohydrate and decreased the rate of accumulation and final concentration of lactic acid and other fermentation products. In addition, DM content might be expected to affect the rate of sugars available to the lactic acid bacteria from the enzymatic breakdown of complex carbohydrates in the plant.

The objectives of this study were to determine the effects of DM level on the fermentation of ensiled alfalfa and the rate of starch hydrolysis during ensiling.

Materials and Methods

Alfalfa, that was mowed, field-wilted and harvested with normal field equipment, was ensiled in mini-silos in each of three harvests (early June, mid-July and late August). In each trial, four DM levels from unwilted to greater than 60% DM were studied. In the third trial, glucose was added to half of the silos in order to compare silage fermentation with and without substrate limitations.

In the first two harvests 30 mini-silos were filled at each DM level; 40 were filled in the third harvest. The mini-silos were sealed and incubated in a water bath at 30°C. For the first two harvests, three mini-silos per treatment were opened, emptied aseptically and analyzed after 1, 2, 3, 4, 5, 7, 10, 14 and 60 d. For third harvest, only 2 mini-silos per treatment were opened on the same schedule.

The initial alfalfa and the silages were analyzed for lactic acid bacteria, reducing sugars, starch, pH, moisture content and fermentation products (lactate, acetate, ethanol, succinate, formate, propionate and butyrate). Initial and final samples were also analyzed for buffering capacity.

Results and Discussion

As in studies with other crops, increasing DM level generally decreased the rate and amount of fermentation and increased final pH. The ratio of the fermentation products varied with DM content. The highest relative lactic acid contents were observed between 40 and 55% DM (Figure 1). The highest relative levels of acetic and succinic acids were in the unwilted silages whereas ethanol was highest in the driest silages. The DM content also affected the shape of the relative lactic acid pro-

duction curve as a function of time of ensiling. These results suggested that the dominant strains of bacteria in fermentation varied with DM level. Additional research into the dominant strains may be beneficial in determining what species are good candidates as inoculant strains at various DM contents.

The highest amounts of starch hydrolysis occurred in the unwilted silages and decreased with increasing DM. However, hydrolysis rates for alfalfa less than 60% DM were unaffected by DM level. Rates in this DM range were proportional to starch concentration (Figure 2) and declined linearly with time. Consequently, the high hydrolysis rates for the unwilted silages were the result of high starch contents at ensiling relative to those in the moderately wilted silages.

The addition of glucose to the two driest silages (51 and 64% DM) in the third trial did not significantly affect final pH or ratio of fermentation products. With wetter silages, glucose addition de-

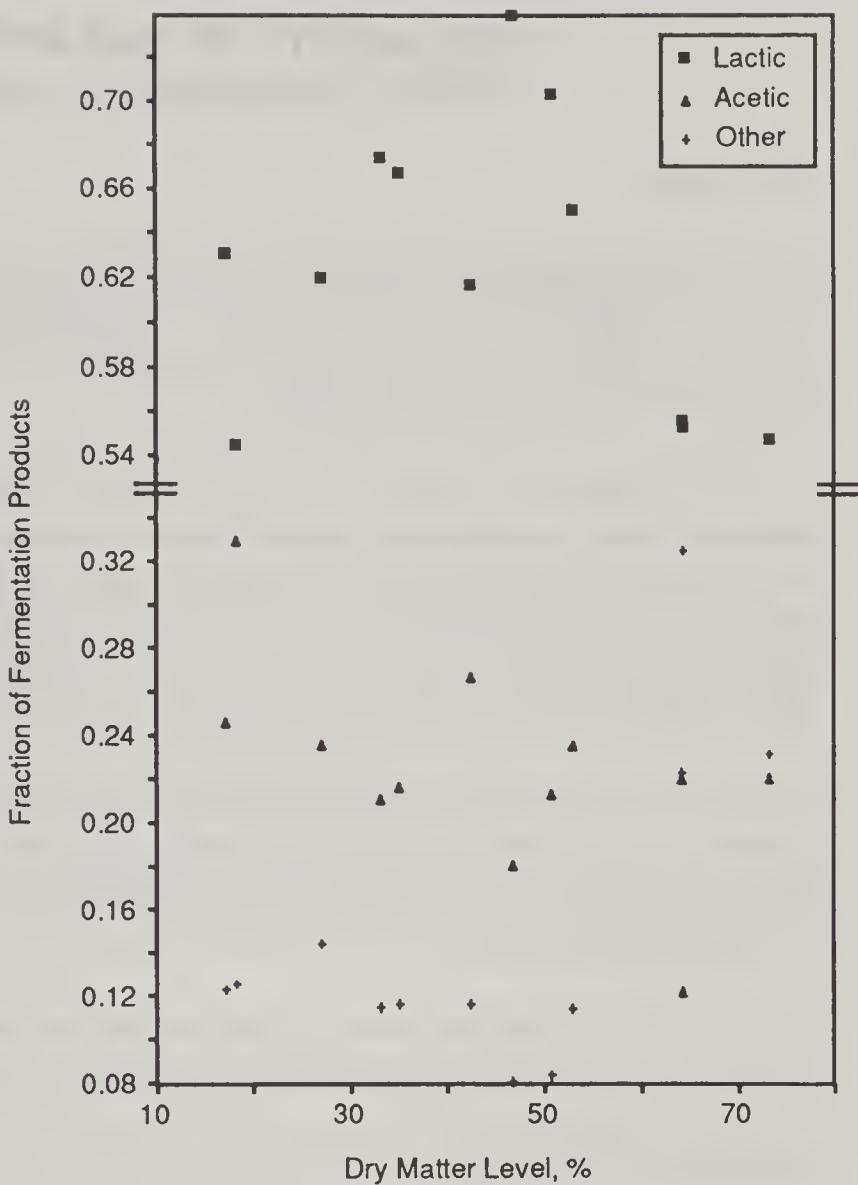


Figure 1. Fermentation products in the silage silages as a fraction of total.

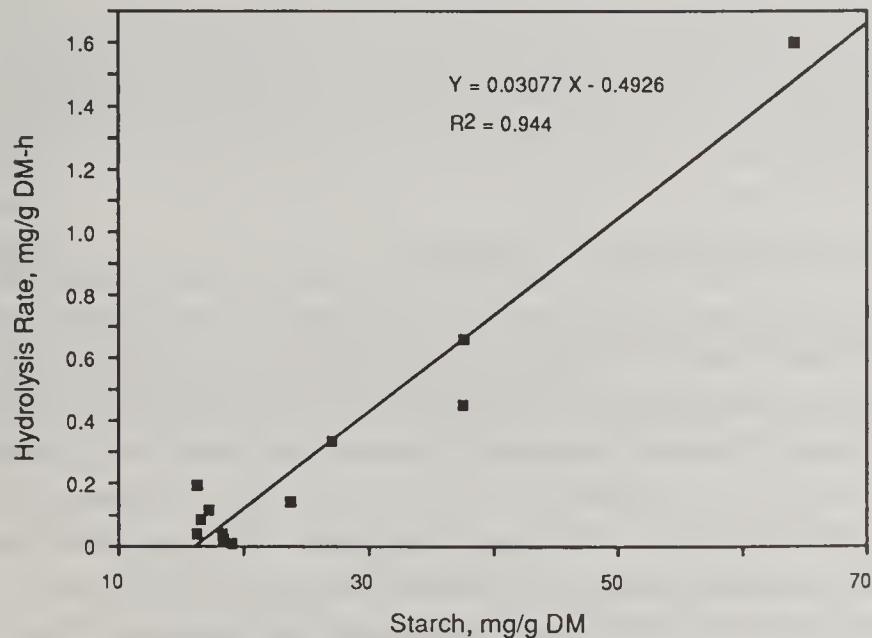


Figure 2. Initial starch hydrolysis rates as a function of starch concentration for all DM levels studied.

creased final pH and shifted the ratio of fermentation products toward lactic acid. Glucose addition also inhibited starch hydrolysis at all DM levels. Since the predominant amylolytic end product in alfalfa is maltose, not glucose, the cause of this result is not simple end product inhibition. However, the practical implication is that the value of sugar addition is actually less than the amount applied. Further research into the mechanism of inhibition is needed to more precisely estimate the value of sugar and enzyme silage additives.

ENSILABILITY OF MAT-PROCESSED ALFALFA

R.E. MUCK, R.G. KOEGEL, K.J. SHINNERS and R.J. STRAUB

Introduction

Losses in harvesting alfalfa with current machinery are substantial. Handling alfalfa as hay results in dry matter (DM) losses of 15 to 22% under typical U.S. conditions whereas losses in harvesting as silage are on the order of 10%. Rainfall can increase those losses by leaching nutrients, shattering leaves and prolonging plant respiration.

In order to reduce these losses, a radically different machine was proposed at the Dairy Forage Research Center to replace the standard mower-conditioner. This machine carries out four processes: mows, macerates (severely conditions) the crop, presses the crop into a continuous mat 6 to 8 mm thick and deposits the mat onto the stubble for drying. Usually, mat-processed alfalfa dries to 80% DM in 6 h or less. Even under poor drying conditions, wilting to normal silage DM's should occur in a few hours, creating a wilted silage system with little or no weather risk.

Studies on the mat-processed alfalfa have shown that it consolidates more quickly than normally-harvested alfalfa, making the mat-processed alfalfa more desirable for packing into bunker silos. In addition, the mat-processed alfalfa is more digestible and has a higher feed intake than normally-harvested alfalfa silage.

The objectives of this study were to compare the rate and extent of fermentation in mat-processed alfalfa with normally-harvested alfalfa with and without the use of a commercial inoculant.

Materials and Methods

Two similar trials were performed on first (June 9) and second (July 14) cutting alfalfa. Alternate swaths were mowed with a mower-conditioner and a prototype mat-making machine. After 3 h, the mat-processed alfalfa was loaded using a belt and tine pickup, taken to a stationary chopper, chopped to a theoretical length of cut (TLC) of 20 and 10 mm, respectively, for first and second cutting, and ensiled. The following day, when the normally mowed alfalfa had reached approximately the same DM content, it was chopped to a TLC of 6 and 10 mm, respectively for first and second cutting, and ensiled.

The alfalfa was ensiled in mini-silos. In the first trial, there were three treatments: mat-processed, normally harvested (control) and normally harvested inoculated with lactic acid bacteria (inoculated control). In the second trial, a fourth treatment, mat-processed with inoculation, was added. The inoculant contained Lactobacillus plantarum and Pediococcus cerevisiae and supplied approximately 5×10^5 colony forming units (CFU)/g alfalfa. For each treatment, 18 mini-silos were filled, sealed and incubated at 30°C in a water bath. At 1, 2, 4, 8, 16 and 32 d, 3 mini-silos of each treatment were removed from the water bath and immediately frozen at -18°C until analyzed. The initial alfalfa and all silage samples were analyzed for pH, moisture content, total Kjeldahl nitrogen, soluble non-protein nitrogen (NPN), free amino acids, ammonia (NH3) and fermentation end products. The initial alfalfa samples were also analyzed for lactic acid bacteria (LAB), total non-structural carbohydrates, reducing sugars and buffering capacity.

Results and Discussion

Mat-processed alfalfa had characteristics which caused it to ensile as well as or better than the control alfalfa. Uninoculated, mat-processed alfalfa fermented quickly, reaching final pH in half the time or less than that of the uninoculated control alfalfa. This was in part due to higher levels of epiphytic LAB on the mat-processed alfalfa at ensiling. However, comparisons between the mat-processed and inoculated control treatments suggest that the mat-making process caused the sugars and other substrates used by the LAB to be more immediately available, increasing fermentation rates.

In both trials, the mat-processed silages had the highest concentrations of fermentation products. Since the levels of sugars and non-structural carbohydrates were similar between the mat-processed and control alfalfa at ensiling, these results suggest that mat processing of the alfalfa increased the breakdown of complex carbohydrates in the plant during ensiling. This could enhance the ensiling of many forages like alfalfa which may have inadequate sugar levels.

In contrast, mat-processed alfalfa was not necessarily more prone to plant proteolytic activity. In the first trial, the mat-processed silage had the lowest NPN level. In the second, the NPN contents were higher in the mat-processed silages; however, there were no statistically significant differences among the four treatments.

Ammonia contents were unaffected in the first trial by treatment. In the second trial, mat-processed silage had significantly higher NH₃ levels. This may have been related to the high initial LAB numbers on the mat-processed alfalfa in that trial. Although NH₃ contents were not overly high, effects on this parameter should be monitored in future studies because of its negative correlation with feed value.

Finally, it appears that inoculation of mat-processed alfalfa is less likely to be beneficial than with normally-harvested alfalfa. Fermentation rates without inoculation in the mat-processed alfalfa were similar to inoculated control alfalfa. Also, the high epiphytic LAB levels suggest that current inoculants would supply insufficient levels of LAB to dominate many of the fermentations. More research is needed to investigate LAB numbers on mat-processed alfalfa and to test various inoculants before conclusive statements regarding their effectiveness on mat-processed alfalfa can be made.

Overall, the mat-processed alfalfa ensiled well, and this study suggests that it has advantages over alfalfa harvested with current machinery.

A COMPREHENSIVE MODEL OF FORAGE CHANGES IN THE SILO

D.R. BUCKMASTER, C.A. ROTZ and R.E. MUCK

Introduction

Evaluation of forage systems and accurate assessment of alternative management strategies or technologies requires a means for estimating losses and quality changes during storage. Tradeoffs between storage losses, feedout flexibility and structure costs must be considered. In order to compare silage systems, a silo model which considers all dry matter losses and quality changes is needed. Such a model would be useful in determining when to repair or replace a silo which is already on the farm and to determine the most economical sizes and types of silos for a given farm. The literature contains several models dealing with silage storage, yet a model which includes the entire ensiling process including filling, fermentation and feedout has not been presented.

The objective of this work was to develop and evaluate a comprehensive yet computer-efficient model that considered both the biological and physical processes involved in ensiling. Specific objectives were 1) to develop a comprehensive model of the ensiling process, 2) to validate the model by comparing simulated and actual data, and 3) to conduct a sensitivity analysis of important parameters of the model.

Materials and Methods

The ensiling process was modeled in 4 phases. The first phase was preseal or before sealing. A silo was filled by plots (in our model, one plot was the material harvested in 3 hours). The first phase was caused by aerobic respiration which occurred until a given plot was covered with another plot or, in the case of the last plot put into the silo, until the silo was covered with plastic. The second phase was fermentation which included all changes occurring in the absence of oxygen. The third phase was infiltration. This phase involved oxygen infiltration through the silo wall (tower silos) or through the cover (bunker silos). The oxygen penetration into the stored material made aerobic respiration possible. The last phase was feedout; dry matter loss due to aerobic respiration on the silage surface inside the silo and in the feed bunk were modeled. The intended use of this model was for wilted silages with dry matter contents above 30%, so effluent production was not considered.

The preseal, infiltration and feedout losses were all modeled based upon theoretical relationships of respiration. Respirable substrate in the forage was lost at a rate dependent upon the oxygen available, the density of the forage and the length of the phase. Fermentation was modeled with empirical relationships which related forage quality following fermentation to forage quality into the silo and other fermentation conditions. The empirical models were developed from data obtained from a detailed mechanistic model of silage fermentation. Fermentation changes were modeled as functions of dry matter content, temperature and air to herbage ratio for corn and alfalfa silages.

A validation of the silo model was done by comparing simulated and actual losses for silos reported in the research literature. Further verification of the model was done by testing the sensitivity of predicted silo losses to changes in various silo parameters.

Results and Discussion

A model was developed and evaluated which described the ensiling process in top and bottom unloaded tower silos and bunker silos. Dry matter was lost as oxygen penetrated into the forage material, and all dry matter lost was respirable substrate (not protein or fiber). Loss during preseal depended upon fill rate and mean respiration rate over the depth of the plot. Rate of loss during infiltration was primarily a function of silage density and silo configuration. Loss during feedout was related to feedout rate and ambient temperature during feeding. Assuming good management practices, total silage losses were about 10% in all types of silos. Infiltration accounted for most of the dry matter loss during ensiling.

The silo model was used to predict dry matter losses and non protein nitrogen concentrations under conditions for which published data were available. Comparisons indicated that the model predicted dry matter loss reasonably well, but lack of complete input data precluded a formal validation. Predictions of non protein nitrogen concentrations agreed reasonably well with published data.

Because most dry matter loss occurred during infiltration, the total loss was most sensitive to silo permeability, silo size and feedout rate. A change in silo wall permeability from 2 to 4 cm/h caused 2.0 and 3.2% more dry matter loss in medium-sized, top and bottom unloaded tower silos, respectively. Increasing the feedout rate caused a reduction in total dry matter loss. Emptying a medium-sized top unloaded tower silo in 120 days rather than 360 days resulted in 4.5% less dry matter loss. An increase in temperature of 26 C caused dry matter loss to increase 1.0 to 3.5 percentage units. Silo capacity also affected dry matter loss (especially in tower silos) because of the increased density and the higher volume to surface area ratio in larger silos. Dry matter loss was relatively insensitive to initial quality because respirable substrate was relatively constant. Similarly, losses during ensiling of corn were similar to those during ensiling of alfalfa. The effect of moisture content on silo losses was not dramatic over the range of 50-65%.

EFFECTS OF A CORN SILAGE INOCULANT ON SILAGE QUALITY AND UTILIZATION BY GROWING HOLSTEIN HEIFERS

C.M. WACEK and L.D. SATTER

Introduction

Benefits from microbial inoculation of corn silage have generally been small or nonexistent. Adequate nutrients and sufficient numbers of natural microflora often insure a successful fermentation. The purpose of this study was to examine the effects of an inoculant produced specifically for corn silage on corn silage quality and nutritive value for growing Holstein heifers.

Materials and Methods

Corn silage at full dent maturity (black layer stage) was chopped to a theoretical length of 1/4 inch and ensiled in two plastic Ag Bags®. Silage consisted of Control (C) silage receiving no additive

treatment, and Treatment (T) silage which was inoculated using AgMaster® corn silage bacterial inoculant containing *Lactobacillus* and *Pediococcus* species. Inoculant was applied to supply 200,000 viable organisms per g of ensiled crop.

Eighty Holstein heifers were divided into 40 light and 40 heavy heifers. Animals were ranked based on weight, grouped into pairs and then randomly assigned to one of the two treatments in a 2x2 factorial design. Initial weights for light and heavy pens averaged 310 and 390 kg, and 313 and 392 kgs for C and T diets, respectively. Animals were weighed for three consecutive days at the start of the experiment and once every two weeks thereafter. Final weights at the completion of the trial were taken for two rather than three consecutive days due to a scale malfunction occurring on day three. Animals were on trial for 86 days. Diets consisted of 90% corn silage, 8.4% soybean meal, .6% dicalcium phosphate and .5% urea with the remainder being minerals and vitamins formulated to meet 1978 NRC requirements for growing Holstein heifers. Diets were fed as total mixed rations once daily to provide a 5-10% refusal rate. Intakes and refusals were recorded daily. The growth trial was analyzed by multivariate analysis using MANOVA in the statistical analysis system (1987).

Results and Discussion

Dry matter content of corn silage at ensiling averaged 47.4% and 48.3% for C and T silage, respectively. Bacterial counts (Rogosa media) at time of ensiling were 1.90×10^7 organisms per g⁻¹ of T corn silage, compared to 5.86×10^7 organisms per g⁻¹ for C silage. Dry matter recoveries from the silo bags averaged 88 and 86% for control and inoculated silages, respectively. Chemical analysis of silages as fed is presented in Table 1.

Heifer performance results are given in Table 2. One heifer was dropped prior to the start of the trial due to an injury and could not be replaced. Average daily gains, dry matter intakes, intakes as a percentage of body weight and feed efficiencies were similar across diets.

Due to large numbers of epiphytic organisms present on the corn silage at ensiling, the inoculant did not appear able to outcomplete the natural microflora. This is evidenced by greater numbers of bacteria on C silage, similar chemical composition across diets as well as similar average daily gains, dry matter intakes and feed efficiency measures of growing Holstein heifers.

Table 1. Chemical composition of corn silage as fed.

Component	Control Silage	Inoc. Silage
Dry Matter (%)	47.7 + 2.9% (DM Basis).....	49.1 + 2.3
Crude Protein	8.7 + .4	8.6 + .4
NDF	39.2 + 1.8	38.6 + 2.7
ADF	21.8 + .6	21.4 + 1.4
ASH	4.0 + .5	3.8 + .7

Table 2. Performance of heifers fed control and inoculated corn silage.

Item	Control Silage		Inoc. Silage		SEM
	Light	Heavy	Light	Heavy	
Number of heifers	20	20	20	19	
Initial weight (kg)	310	390	313	392	
Final weight (kg)	393	476	393	481	
Average daily gain (kg)	1.06	1.09	1.02	1.13	.169
Dry matter intake (kg/d)	9.11	10.37	9.13	10.52	
Intake (% of BW)	2.59	2.39	2.59	2.41	
Feed efficiency (kg DM/kg gain)	8.59	9.51	8.95	9.13	

EFFECT OF BACTERIAL INOCULANTS AND CELLULASE ON QUALITY OF ALFALFA SILAGE

C.M. WACEK, J.A. WOODFORD and L.D. SATTER

Introduction

Alfalfa, due to its high buffering capacity and relatively low concentration of fermentable substrate, is considered more difficult to ensile than other forage and cereal crops. Populations of homofermentative lactic acid bacteria, critical for an efficient and rapid fermentation, can be low in legume silages under certain conditions. Under these conditions bacterial inoculants that provide viable lactic acid bacteria in numbers sufficient to improve silage fermentation, and cellulase enzymes to increase the amount of fermentable substrate, may support a more desirable fermentation and consequently improve animal performance. One objective of this study was to determine the influence of bacterial inoculation on the fermentation of high moisture silage and its subsequent utilization by lactating dairy cattle. A second objective was to determine if inclusion of a cellulase enzyme with a bacterial inoculant could improve response to inoculation.

Methods and Procedures

First cutting alfalfa was harvested and ensiled in four horizontal concrete bunker silos as control (C) silage or as one of three treatment silages. Treatments consisted of a combined addition of Great Lakes microbial inoculant and Finnsugar cellulase enzyme (GL+E), Great Lakes (GL) microbial inoculant alone and Qualitech (Q) microbial inoculant. To monitor fermentation characteristics, core samples were removed on days 1, 2, 4, 8, 16 and 30. Forty eight lactating Holstein cows averaging 14 weeks postpartum were assigned to treatments in a 4x4 latin square design. Periods were 21 days in length. Body weights were taken and milk samples analyzed for fat and protein during the last week of each period. Diets were composed of 60% silage, 27% high moisture ear corn and 12% soybean meal. Diets were fed once daily as total mixed rations to provide 5 to 10% refusals. Intakes and feed refusals were recorded daily.

Results and Conclusions

Dry matter content of alfalfa at harvest averaged 36.4%. Crude protein content of silage averaged 16.9% and ADF and NDF averaged 38.0% and 44.9%. Inoculation of alfalfa resulted in higher counts of lactic acid bacteria. Epiphytic bacteria on C silage averaged 1.01×10^4 organisms g⁻¹, while addition of GL inoculant resulted in an average of 5.01×10^4 organisms g⁻¹ and addition of Q inoculant resulted in 3.67×10^5 g⁻¹. Treated silages showed more rapid rates of pH decline and lower final pH compared to C silage. Organic acid and ethanol contents of alfalfa during feedout are presented in Table 1. Lactic acid was significantly higher for the GL+E silage than for the GL silage. Acetic acid content was significantly different among all treatments and was highest for the C silage and lowest for the Q silage. Lactic acid:acetic acid ratios for C, GL+E, and GL and Q silages were 2.08, 4.74, 3.41 and 5.55, respectively. Other organic acids and ethanol occurred in relatively minor amounts, with significant differences in succinic acid content ($P < .058$) between treatments and a slightly lower content of ethanol in the Q silage. Non-protein nitrogen, ammonia-nitrogen, and free amino acid-nitrogen data for silages during feedout are presented in Table 2. All treatments experienced significantly less proteolysis ($P < .004$) during feedout than C silage. Non-protein nitrogen, as a % of total nitrogen, averaged 46.4% for C and 40.8% for treated silages. Ammonia-nitrogen and free amino acid-nitrogen as a % of total nitrogen was also reduced with treatment.

Performance of lactating cattle, fed silage based diets, is presented in Table 3. There were no significant differences between treatments in DM intake of diets, milk production parameters or milk components. There was a significant difference in body weight change between control diets and treatment diets. Treatment diets supported a gain of 16.4 kg compared to a 5.2 kg loss (over the length of the experiment) for cows fed the control diet.

Table 1. Organic Acid and Ethanol Content of Alfalfa Silage During Feedout

	C ¹	CL+E ¹	GL ¹	Q ¹	SE	P ²
.....mg/g DM.....						
Lactate	81.5 ^{AB}	84.9 ^A	74.7 ^B	82.2 ^{AB}	4.7	.069
Acetate	26.5 ^A	17.9 ^C	21.9 ^B	14.8 ^D	1.04	<.001
Propionate	1.24 ^A	.41 ^{AB}	.54 ^{AB}	.35 ^B	.528	.129
Butyrate	1.50	.69	.97	.68	.576	.222
Succinate	5.66 ^A	3.09 ^C	4.04 ^B	2.72 ^C	.450	<.001
Ethanol	5.01 ^A	3.97 ^{AB}	4.84 ^A	3.60 ^B	.713	.058

¹C=Control, GL+E=Great Lakes inoculant and Finnsugar enzyme, GL= Great Lakes inoculant, Q=Qualitech inoculant.

²Probability of significant treatment effect.

^{ABCD}Means within rows with unlike superscripts differ at P value shown.

Table 2. NPN, ammonia, and free amino acid content of silage during Feedout.

	C ¹	GL+E ¹	GL ¹	Q ¹	SE	P ²
NPN, mg/g DM	12.57 ^A	11.41 ^B	10.71 ^B	10.94 ^B	.669	.015
NPN, % Total N	46.4 ^A	42.2 ^B	40.0 ^B	40.3 ^B	1.89	.004
Ammonia-N, % of total N	9.2 ^A	7.0 ^C	7.8 ^B	6.6 ^C	.431	<.001
Free Amino acid-N, % of total	25.5 ^A	23.3 ^B	23.1 ^B	23.8 ^{AB}	1.07	.040

¹C=Control, GL+E=Great Lakes inoculant and Finnsugar enzyme, GL= Great Lakes inoculant, Q=Qualitech inoculant.

²Probability of significant treatment effect.

^{ABC}Means within rows with unlike superscripts differ at P value.

Table 3. Average daily DM intake, milk production, and BW change of lactating cows.

	C ¹	CL+E ¹	GL ¹	Q ¹	SEM
DM intake, kg/day	19.7	19.7	19.4	19.4	1.4
Milk, kg/day	27.19	27.06	26.85	27.30	1.68
4% FCM, kg/day	25.15	24.96	24.95	25.07	1.68
Milk Fat %	3.53	3.52	3.55	3.48	.18
Milk CP %	3.03	3.03	3.03	3.03	.09
BW,kg	594	595	600	602	26
BW change, kg ²	-5.2 ^A	16.3 ^B	16.3 ^B	16.7 ^B	30.7

¹C=Control, GL+E=Great Lakes inoculant and Finnsugar enzyme, GL=Great Lakes inoculant, Q=Qualitech inoculant.

²Mean with different subscripts differ, P<.05.

²Represents the cumulative weight change attributed to treatments over the whole experiment.

EFFECTS OF POTASSIUM SALTS AND CITRIC ACID ON FERMENTATION OF ALFALFA SILAGE

W.L. SHOCKEY and A.L. BARTA

Introduction

Rapid attainment of low forage pH is essential for preservation of crops as silage. This is particularly true for hay crop silages that have a high protein content. Plant proteolytic enzyme activity remains active until the pH drops to about 4.5, depending upon moisture content and other factors.

Previously, researchers postulated that high concentrations of K, Ca, and Mg could increase the time required to attain low pH by forming salts and buffer systems with fermentation end products. Initial experiments with greenhouse grown alfalfa indicated that high concentrations of alkaline minerals were associated with fast rates of pH decline. Results of a second experiment in which KCl

was added to alfalfa and ensiled into experimental silos indicated that the faster rate of pH decline observed in the greenhouse experiment was a result of changes in plant chemistry caused by fertilization, not alkaline mineral content per se.

A large proportion of plant K is present as potassium citrate (K-citrate). Citric acid can be fermented by lactic acid bacteria. Therefore, an experiment was conducted to compare the effects of K-citrate and citric acid to KCl addition on alfalfa silage fermentation. The hypothesis was that K addition in a form more closely representative of the naturally occurring form of K would affect the alfalfa fermentation differently than K added as the Cl salt.

Materials and Methods

Third cutting alfalfa was cut, wilted, and chopped (2-3 cm). Because of high temperatures and drought conditions prevalent at the time of harvest forage was cut as late as feasible in the afternoon (1500-1700 h) and chopped as early as feasible the next morning (0800-1000 h). Even with this short wilting time and the presence of dew, average dry matter content was 57%. As will be seen in results section this low moisture forage did not ferment well.

Chopped forage was divided into four piles and no additive or KCl, K-citrate, and citric acid were added (36, 50, and 32 g/kg wet forage, respectively). The piles were mixed by hand and ensiled into 24 (3 days, 4 treatments, 2 replicates) 60 x 10 cm PVC pipes for 0, 3, 6, and 28 days. When silos were opened the forage was mixed and immediately a subsample was taken for microbiological analysis and another subsample was taken for dry matter determination (100 C). The remaining sample was frozen for subsequent lyophilization. Analyses completed were dry matter, total anaerobes, pH, nitrogen, protein nitrogen, 2-amino nitrogen, starch, and soluble sugar.

Results and Discussion

Lack of pH decline, decrease in starch after day 0, and increase in soluble sugar after day 0 (Table 1) indicates that little or no fermentation occurred. Dry matter content was 57% and nitrogen concentration on dry matter basis was 3.41% in untreated forage. Because of drought conditions that prevailed during the harvest of this forage, the dry matter content was about 20 percentage units higher than planned even though an effort was made to minimize wilting time. Evidently, forage should have been direct cut or wilted for only a few hours rather than overnight. The overly dry forage was likely the main reason for lack of fermentation.

Despite the lack of fermentation, citric acid and K-citrate addition slowed the rate of proteolysis as measured by TCA precipitable nitrogen (Table 1). All the proteolytic activity occurred within 3 days in control and KCl treated forage. For forage treated with K-citrate or citric acid proteolysis was not complete until day 6. Breakdown of protein fragments as estimated by 2-amino nitrogen content indicates that all additives slowed breakdown of proteolytic products, citric acid being most effective. Reduced pH caused by citric acid addition probably accounted for the lowered 2-amino nitrogen content.

Also indicative of slow or nonexistent fermentation is the time required for maximum number of anaerobes to appear. In silages of more normal moisture content, maximum anaerobe counts appeared by day 3 to 5. In this experiment maximum counts occurred on days 6 and 28 (Table 1).

Although some useful information was derived from this experiment it will be repeated next year using forage with a higher moisture content (35 to 45% dry matter).

Table 1. Effect of K salts and citric acid on fermentation of alfalfa silage.

Day	Control	KCl	K-citrate	Citric acid	Mean
pH					
0	5.79	5.72	6.13	4.14	5.44
3	5.70	5.68	6.07	4.07	5.38
6	5.68	5.67	5.99	4.40	5.43
28	5.61	5.64	5.96	4.37	5.39
STARCH (% DM)					
0	4.09	3.57	3.68	3.04	3.59
3	3.29	3.40	3.03	2.67	3.10
6	3.61	3.23	2.93	2.82	3.15
28	3.41	3.10	2.96	2.82	3.07
SOLUBLE SUGAR (%DM)					
0	6.43	6.49	6.56	6.49	6.49
3	7.46	7.49	7.09	7.07	7.27
6	7.97	7.28	7.55	7.04	7.46
28	7.56	7.49	7.79	7.47	7.58
-TCA PRECIPITABLE NITROGEN (% TOTAL N)-					
0	80.9	85.0	83.9	77.4	81.8
3	51.8	61.7	71.5	72.1	64.3
6	57.8	63.6	57.1	60.9	59.8
28	55.3	59.2	58.7	69.1	60.6
-2-AMINO NITROGEN (% TOTAL N)-					
0	.54	.56	.48	.50	.52
3	1.36	1.11	1.06	.85	1.09
6	1.61	1.26	1.40	1.02	1.32
28	2.22	1.82	1.82	1.32	1.79
LOG ₁₀ ANAEROBES/g WET FORAGE					
0	5.73	5.20	5.15	4.71	5.20
3	6.42	6.41	5.71	5.60	6.03
6	6.97	6.43	6.70	6.64	6.68
28	7.08	6.07	6.89	5.42	6.36

EFFECT OF NaCl ON FERMENTATION OF ALFALFA SILAGE INOCULATED WITH CLOSTRIDIA spp. AND LACTIC ACID BACTERIA

W. L. SHOCKEY

Introduction

Sodium chloride (NaCl) has been used as an additive to preserve and pickle food from time immemorial. Logically, NaCl may also inhibit the growth of undesirable silage microorganisms; however, use of NaCl as a silage additive has not yielded successful results. Silage bacteria activity, pH, seepage, and mold growth have been shown to be unaffected by NaCl addition.

Preliminary experiments with pure culture bacteria indicated that small amounts of NaCl inhibited the growth of Clostridia butyricum while having little affect on the growth of Lactobacillus plantarum or Streptococcus faecium. An experiment was conducted to determine if NaCl could selectively inhibit growth of Clostridia on direct cut alfalfa.

Materials and Methods

Fourth cutting alfalfa was cut by hand into 2-3 cm pieces, well-mixed, then divided into five piles and treated as follows: 1) 1.25 ml substrate depleted media (SDM)/100 g wet forage; 2) .63 ml SDM + .63 ml Clostridia culture/100 g wet forage; 3) Treatment #2 + 4 g NaCl/100 g wet forage; 4) .63 ml Clostridia culture + .63 ml lactic acid bacteria culture/100 g wet forage; and 5) Treatment #4 + 4 g NaCl/100 g wet forage. For each treatment, forage was packed into 8, 20 x 150 mm test tubes (20 g per tube). Tubes were purged with CO₂ for 1 min, tightly stoppered, and placed into a metal spring test tube holder to allow fermentation gases to "burp". The experiment was replicated 3 times.

After 3, 7, 21, and 60 days two tubes from each treatment were opened, the contents mixed, 20 g used for microbial analysis, and 20 g used for pH and organic acid analysis. Analyses completed are dry matter of green herbage, total anaerobes, pH, and organic acids.

Results and Discussion

Dry matter content of alfalfa was 19.3, 23.6, and 31.6% for replicate 1, 2, and 3, respectively. An-aerobe, pH, and acid data (Table 1) for the control indicate that the forage underwent a satisfactory fermentation using this system.

The pH did not decline rapidly or to a low level for either Clostridia or Clostridia + NaCl. Microbiology and acid data indicates that for the Clostridia treatment a larger number of bacteria were present compared to the control and that even though the forage was inoculated with Clostridia only, the epiphytic lactic acid bacteria dominated early in the fermentation. However, the increasing amount of butyric acid at day 60 compared to day 21 is an indication that the Clostridia may have dominated had the experiment been followed beyond 60 days.

Salt effectively inhibited Clostridial activity as evidenced by the lack of butyric acid produced in the Clostridia + NaCl treatment. Even though salt is generally not as inhibitory to lactic acid bacteria as it is to Clostridia, only about half as much lactic acid was produced compared to control and Clostridia treatments. Evidently, salt inhibited the epiphytic lactic acid bacteria population.

Inoculation with lactic acid bacteria (Lactobacillus plantarum and Streptococcus faecium) effectively inhibited Clostridia growth with or without added NaCl and resulted in a rapid, nearly immediate drop in pH with high levels of lactic acid produced. Although NaCl may have inhibited epiphytic lactic acid bacteria, the species that were added as an inoculant were little affected.

Conclusions

Salt (NaCl) effectively inhibited growth of Clostridia and, apparently, epiphytic lactic acid bacteria. High final pH and low levels of lactic acid indicate that salt treated forage was not well preserved

and supports the observations seen in early studies of salt addition to hay crop silage. Inoculation with lactic acid bacteria was more effective than salt in inhibiting Clostridia growth and contributing to a well preserved silage.

Table 1. Effect of NaCl, Clostridia, and lactic acid bacteria on fermentation of alfalfa silage¹.

DAY	CTR	CLOS	CL+NA	CL+LA	CL+NA+LA	MEAN
pH						
0	5.78	5.80	5.70	5.83	5.80	5.78
3	5.71	5.47	5.69	4.40	4.58	5.17
7	5.27	5.35	5.76	4.34	4.48	5.04
21	5.18	5.11	5.39	4.36	4.40	4.89
60	4.88	5.23	5.16	4.36	4.37	4.80
LOG_{10} ANAEROBES/g WET FORAGE						
0	4.88	4.77	3.90	6.66	6.63	5.37
3	7.79	8.13	7.37	9.06	8.71	8.21
7	6.99	8.31	6.28	8.02	8.15	7.55
21	6.11	6.31	6.80	7.01	5.58	6.36
60	6.04	6.40	5.72	5.14	4.83	5.63
LACTIC ACID (% DRY MATTER)						
0	.40	.40	.83	.67	.46	.54
3	3.35	3.35	.54	8.05	9.21	4.91
7	5.28	3.91	.04	11.65	8.86	5.95
21	6.05	6.78	2.56	12.85	10.38	7.72
60	6.76	5.37	1.66	11.75	10.88	7.28
ACETIC ACID (% DRY MATTER)						
0	.19	.27	.21	.21	.25	.23
3	.42	.69	.25	.54	.35	.44
7	.81	1.33	.21	.67	.40	.69
21	.73	1.27	.25	.79	.48	.71
60	1.16	1.29	.79	.83	.58	.94
BUTYRIC ACID (% DRY MATTER)						
0	.04	.04	.02	.00	.02	.02
3	.10	.35	.06	.06	.10	.15
7	.15	.49	.08	.04	.04	.17
21	.10	.69	.02	.02	.02	.17
60	.49	1.79	.02	.04	.04	.49

¹CTR=CONTROL; CLOS=CLOSTRIDIA; CL+NA=CLOSTRIDIA+NAACL;
CL+LA=CLOSTRIDIA+LACTIC ACID BACTERIA;
CL+NA+LA=CLOSTRIDIA+NAACL+LACTIC ACID BACTERIA.

FORAGE UTILIZATION BY CATTLE AND SHEEP

FISH MEAL VS. SOLVENT SOYBEAN MEAL FOR LACTATING COWS FED ALFALFA SILAGE AS SOLE FORAGE

G.A. BRODERICK

Introduction

Most studies comparing protein supplements which are resistant to ruminal degradation have used corn silage as forage because its low crude protein (CP) content allows diets to be formulated with greater amounts of test protein. However, there is evidence that despite high CP levels, the protein in legume forages is poorly utilized by ruminants. This is particularly true for alfalfa silage, and resistant proteins should be tested as supplements for that forage. Cows fed alfalfa silage or hay as sole forage produced less milk protein and milk with lower protein content than cows fed corn silage-based diets supplemented with soybean meal to equalize dietary CP (Broderick, *J. Dairy Sci.* 68:3262, 1985).

Fish meal is known to be substantially resistant to ruminal degradation. The purpose of this study was to determine if the protein in fish meal (FM) would be used more efficiently than that in solvent soybean meal (SBM) in lactating dairy cows fed alfalfa silage-based diets.

Materials and Methods

Twenty Holstein cows, averaging 583 kg, lactation number 2.3, 53 d in milk, and 39.7 kg/d milk, were blocked into two groups according to production and stage of lactation. Supplements of about 450 g CP/d from either FM or SBM were fed in a switch-back experiment. Fish meal was a gift from Zapata-Haynie Co., Hammond, LA. Rate and extent of rumen *in vitro* degradation of protein in FM were .031/h and 40%, and in SBM were .096/h and 63%. Supplements were fed for periods of three wk before switching; the complete switch-back cycle totaled six wk. Data were analyzed from the last two wk of each period. Diets were fed *ad libitum* as total mixed rations. Silage was second cutting alfalfa, chopped to a theoretical length of 1.0 cm and stored in a bunker silo. Alfalfa silage was 39% DM as-fed and provided 70% of diet DM (Table 1). Milk production was measured at each milking; milk samples were analyzed for fat, protein, lactose and urea. Cows were weighed on three consecutive d at the start of the trial and at the end of each period. Four h after feeding on d 20 of each period, blood samples were taken from the tail vein of each cow. Blood plasma was prepared and analyzed for glucose and urea.

Results and Discussion

Compared to SBM, FM significantly ($p < .05$ or smaller) increased weight gain, and production of milk, 3.5% FCM, protein and lactose, and protein concentration, and slightly reduced lactose concentration (Table 2). Also, there was a trend for increased fat production. Milk production improve-

ment was small, but very consistent, indicating a modest protein deficiency on the basal diet. Highly significant increases in milk protein concentration and secretion are indicative of more efficient utilization of protein in FM relative to SBM. At 3% protein in milk, the .06 kg protein/d increase corresponds to about 2 kg/d of milk; this is greater than the 1.1 and 1.3 kg/d improvements in actual milk and FCM observed in the study. The nonsignificant difference of .3 kg/d of DM intake cannot account for the .53 kg/d greater weight gain with FM supplement. Increased intake would have provided only about .5 Mcal of the 2.71 Mcal NE₁ required for this extra gain.

Although blood glucose and urea were not influenced by protein, milk urea was higher with supplemental FM (Table 2). The very high urea concentrations in blood and milk reflect the high CP content of the diet (Table 1).

Findings from this study indicate that greater ruminal escape results in more efficient utilization of FM than SBM protein in lactating dairy cows fed alfalfa silage-based diets.

Table 1. Diet composition

Component	Soybean meal	Fish meal
.....(%) of DM).....		
Alfalfa silage	69.7	69.8
High moisture corn	25.1	25.1
Ground shelled corn	—	1.0
Soybean meal	4.3	—
Fish meal	—	2.9
Trace mineral salt	.45	.44
Monosodium phosphate	.41	.72
Vitamin A,D &E con.	.11	.11
Crude protein	20.3	20.1

Table 2. Effect of supplemental protein from solvent soybean meal or fish meal on DM intake, weight gain, production of milk and milk components, plasma glucose and urea, and milk urea.

Item	Supplemental Protein		
	Solvent SBM	Fish Meal	Probability
DM intake, kg/d	22.9	23.2	.296
Weight gain, kg/d	.55	1.08	.030
Milk, kg/d	36.0	37.1	.002
3.5% FCM, kg/d	34.6	35.9	.014
Fat, kg/d	1.18	1.23	.066
Fat, %	3.29	3.33	.500
Protein, kg/d	1.02	1.08	<.001
Protein, %	2.83	2.92	.010
Lactose, kg/d	1.88	1.92	.041
Lactose, %	5.22	5.18	.018
Plasma glucose, mg/dl	59.1	59.9	.812
Plasma urea, mM	7.52	7.58	.861
Milk urea, mM	7.32	7.52	.011

RELATIVE RUMINAL DEGRADABILITY OF PROTEIN IN ALFALFA HARVESTED AS STANDING FORAGE OR HAY

G.A. BRODERICK, S.M. ABRAMS and C.A. ROTZ

Introduction

The protein in alfalfa forage is poorly utilized by ruminants because it is extensively degraded in the rumen. Degraded protein is useful to the animal through the synthesis of protein by the ruminal microbes. However, the amount of microbial protein formed is a function of the energy rather than protein content of the diet, and forages are generally low in digestible energy. There is a major trend toward increased feeding of alfalfa silage to lactating dairy cows. It is not known whether there are substantial differences between alfalfa silage and hay in ruminal protein degradation. The 1988 NRC Dairy Bulletin puts the rumen escape value of alfalfa silage and hay at 23 and 28%, respectively, suggesting a slight advantage to hay. Samples of alfalfa forage, harvested over two seasons as standing forage or hay, were available from a hay-conditioning study. Assuming that the protein degradability of standing forage is similar to that of silage, we used this sample set to obtain inference into whether there are possible differences between hay and silage in ruminal protein escape.

Materials and Methods

A total of 89 samples of alfalfa forage were obtained over the 1984 and 1985 harvest seasons. Samples were harvested at various maturities at all three cuttings during each year. The forage was cut and mechanically conditioned using conventional methods. Half of the forage samples were harvested as Standing Crop by removing bunches of the forage from windrows, freezing immediately in liquid nitrogen, then freeze-drying at a later time. The balance of the forage was allowed to field cure and was harvested as baled hay. The other half of the samples were collected as Hay by coring bales after the hay was put into storage. Samples were ground through a Udy mill and analyzed for DM, total N and ADIN. Ruminal degradation rates were determined using the inhibitor *in vitro* system (Broderick, Brit. J. Nutr. 58:463, 1987). Ruminal degradation rates and estimated escapes were corrected for indigestible N based on ADIN content.

Results and Discussion

There were small differences in N fractions among the samples of Standing Crop and Hay. During 1984, total N was greater in Hay than Standing Crop, and ADIN was greater in Hay than Standing Crop during 1985 (Table 1). The proportion of total protein which was degradable (i.e., not part of the NPN or ADIN fractions) was 2-5% smaller in Hay than Standing Crop. There were no apparent differences in degradation due to cutting, maturity within cutting, or year of harvest. The rate of degradation was faster and estimated ruminal protein escape was lower for Standing Crop than Hay, with essentially the same results each year (Table 1). It is not known if these data are generally applicable to alfalfa silage and hay. However, if it is assumed that the Standing Crop is indicative of alfalfa silage, and our samples are characteristic of alfalfa hay, these results suggest that the ruminal escape value of hay protein would be about 60% greater than silage. The assumption that degradation of protein in Standing Crop represents that of silage may actually be conservative because extensive autolysis of protein occurs in the silo, resulting in high NPN levels in alfalfa silage. Our data imply a substantial protein advantage to harvesting alfalfa as hay rather than silage. This result requires more thorough investigation.

Table 1. Comparison of nitrogen composition and rumen in vitro degradation data for alfalfa forages harvested as standing crop or hay.

Year	Item	Standing Crop	Hay	P
1984	Total N, % of DM	2.48(.27)	2.90(.25)	.004
	ADIN, % of total N	4.6(.9)	5.0(.8)	.401
	Degradable protein (B), %	89.9(1.7)	87.6(2.2)	.023
	Degradation rate (k_d), h ⁻¹	.166(.020)	.077(.030)	<.001
	Estimated protein escape ¹ , %	24(2)	40(9)	<.001
1985	Total N, % of DM	2.65(.55)	2.91(.40)	.372
	ADIN, % of total N	4.5(1.3)	6.0(.9)	.035
	Degradable protein (B), %	90.4(1.9)	85.7(2.0)	.002
	Degradation rate (k_d), h ⁻¹	.179(.053)	.072(.014)	<.001
	Estimated protein escape ¹ , %	24(5)	39(4)	<.001

¹Estimated protein escape, % = $[k_p/(k_p + k_d)] \times B$, where $k_p = .06h^{-1}$.

TRUE ABSORPTION OF CALCIUM AND PHOSPHORUS FROM CORN SILAGE FED TO PREGNANT NONLACTATING DAIRY COWS

F.A. MARTZ and A.T. BELO

Introduction

There were no values found in the reported literature for the true absorption of calcium and phosphorus from corn silage when fed to dairy cows. True absorption is the amount of a mineral which is available to the cow for various body functions. True absorption values and an understanding of absorption of minerals from forage feedstuffs will aid nutritionists in developing improved nutritional programs for dairy cattle.

Materials and Methods

The experimental design was a cross-over with two dietary treatments and four cows with repeated measures on cows. The diet treatments were two levels of calcium and phosphorus.

Each of two periods consisted of a 2-week adjustment period and 1 week of collection. During the collection week total collection and sampling of feces and urine were performed. On day 1 of each collection period each cow was dosed intravenously with 0.75 mCi calcium-45 and 1.5 mCi phosphorus-32 through an indwelling venous cannula.

Cows were housed in a stanchion metabolism unit with individual plywood feed boxes and double rubber mats over a concrete floor. Feces was collected in plastic lined pans beneath a floor grate and

urine was collected by placing a urine collection cup over the cows' vulva and draining the urine to a plastic container beneath the floor.

Formulation of experimental diets is shown in Table 1. The high calcium diet contained 89% corn silage which was diluted with 10% corn cobs to get a calcium level in the diet which would be below the cows' required level. For the low calcium level corn silage was further diluted with corn cobs and hominy grits.

Feed, feces, urine and plasma were digested in strong acid to a clear liquid. Radioactivity was counted in a Beckman LS 7500 Liquid Scintillation Counter. Stable calcium was measured by atomic absorption and stable phosphorus was measured by spectrophotometry using the Molybdo-Vanadate method and a Model DU-65 Beckman Spectrophotometer. A National Bureau of Standards sample of citrus leaves was used to insure accuracy of analysis.

Statistical analysis was done by using the General Linear Models procedure of Statistical Analysis Systems.

Results and Discussion

Calcium and phosphorus balance and true absorption is shown in Table 2. Cows consumed 83% of their NRC calcium requirement from the high calcium (HC) diet and 41% from the low calcium (LC) diet. We wanted the calcium intake to be below required levels to insure that the cows would have maximal absorptive capacity. True absorption of calcium from the LC diet was higher (67.9%) (not statistically significant) than from the HC diet (52.5%). Even though the percent true absorption was higher for diet LC, the actual calcium absorbed was 10.9 g/d compared to 16.9 g/d for the HC diet. Since the calcium intakes were below required levels, the fact that the true absorption values were not statistically different is consistent with the expected response because decreases in true absorption with increasing calcium intake would only be expected at levels above the required intake.

Cows consumed 82% of their NRC required amount of phosphorus from diet HC and 48% from diet LC. There was no significant difference in percent true absorption of phosphorus from the diets. The true absorption values agree with some values in the literature for other forage feedstuffs which range from 81 to 91% but are higher than the 65% true absorption for phosphorus observed in our laboratory for alfalfa and tall fescue fed to lactating cows.

Table 1. Formulation of experimental diets (DM basis).

Ingredient	Diets	
	High calcium	Low calcium
Corn silage	89.0	50.0
Corn cobs, ground	9.8	33.0
Hominy grits	—	15.5
Urea	0.7	1.0
Mineral Supplement	0.5	0.5

Table 2. Calcium and phosphorus balance and true absorption.

Parameter	High calcium	<u>Diets</u>
		Low calcium
Calcium		
NRC Req., g/d	39	39
Intake, g/d	32.2	16.0
Fecal, g/d	21.1	9.11
Urine, g/d	1.3	.66
Endogenous, g/d	5.84	3.97
Balance, g/d	3.96	2.26
Appt. absorbed g/d	11.1	6.89
True absorbed %	52.5	67.9
Phosphorus		
NRC Req., g/d	24	24
Intake, g/d	19.8	11.6
Fecal, g/d	3.19	.46
Urine, g/d	.04	.02
Endogenous, g/d	.58	.40
Balance, g/d	15.99	10.72
Appt. absorbed, g/d	16.61	11.1
True absorbed %	86.9	99.0

TRUE ABSORPTION OF CALCIUM AND PHOSPHORUS FROM ALFALFA AND CORN SILAGE FED TO LACTATING COWS

F.A. MARTZ

Introduction

Very little information exists about the true absorption of calcium and phosphorus from the forage fraction of rations which are fed to high producing dairy cows. A major portion of the reported true absorption values are for mixed rations and/or non-lactating beef cattle. How well a cow absorbs minerals from particular feeds may determine whether the ration is adequate or not.

Materials and Methods

The experimental design was an extra-period cross-over with two dietary treatments, four experimental cows and repeated measures on the same experimental unit (cow). Dietary treatments were alfalfa (A) and alfalfa-corn silage (A-CS).

Each period consisted of a 14-day adaptation period and a 7-day sample collection period. Feed intake, feces, urine, and milk output were measured during the collection period. Each cow was dosed intravenously with Ca^{45} and P^{32} on day 1 of each period. Blood was sampled at varying time intervals during the subsequent 6 days.

Four cows, in lactation 5 and 6, which were producing 38 to 42 Kg of milk daily were randomly assigned to the treatments. The cows had been lactating approximately 60 to 90 days and were fed a ration at the dairy farm prior to this trial consisting of corn silage, alfalfa hay, soy hulls, whole cotton seeds and concentrate. Their calcium and phosphorus intake was calculated to be 150 and 75 g, respectively.

Treatment diets (Table 1) were formulated to be equal in energy, protein, vitamins and minerals except calcium and phosphorus. Rations were also formulated so that a major portion of the calcium and phosphorus came from alfalfa or alfalfa and corn silage. Corn cobs, hominy grits and corn gluten meal were used as sources of fiber, energy and protein and were relatively free of calcium and phosphorus.

Results and Discussion

The average body weight of cows for the trial was 645 kg. Average dry matter intake, apparent ration digestibility, and fat corrected milk production are given in Table 2 for the collection periods. Cows consumed significantly more of the alfalfa-corn silage (A-CS) ration (35.2 vs 32.0 kg/d) than the alfalfa ration (A). This result is probably a reflection of the lower DM digestibility of the A ration than A-CS (62.2 vs 74.5 %). Production of fat corrected milk was higher for ration A-CS than A (35.2 vs 32.0 kg/d) which is consistent with the DM digestibility and the DM intake of the rations. Content of milkfat, protein and somatic cells were not significantly affected by the treatments.

The strategy for this trial was to have cows consume 90% of their calcium and phosphorus requirement so they would be metabolizing at near maximum levels. Cows consumed 104.1 and 122.6 g/d calcium from diets A and A-CS respectively (Table 3). This intake was 86.3 and 93.9 % of the current NRC requirements for calcium. More calcium was consumed from diet A-CS than A which was a reflection of a higher DM intake and slightly higher calcium concentration in diet A-CS.

Calcium absorption was affected by ration (Table 3). Endogenous fecal calcium did not differ by diet but true absorption for calcium from diet A-CS was greater (45.2 vs. 24.0 %) than from diet A.

The cows consumed 31.5 and 48.1 g/d phosphorus (Table 4) from diets A and A-CS, respectively. These levels were 40.6 % of the current NRC requirement for diet A and 57.6 % for diet A-CS. Phosphorus balance was less negative (-5.0 vs. -12.1) for the A-CS compared to the A ration. The improvement in phosphorus balance for ration A-CS over A probably reflects the higher phosphorus intake for A-CS. The percent true absorption of phosphorus from both diets was similar (64.2 vs. 74.2 %).

The partial true absorption of calcium and phosphorus for alfalfa and corn silage is shown in table 4. Partial true absorption was calculated by first calculating a partial true absorption for the calcium or phosphorus in alfalfa for the alfalfa diet. Then that partial true absorption value was applied to the alfalfa in the corn silage diet to calculate the partial true absorption of calcium or phosphorus from corn silage.

The partial true absorption for calcium in alfalfa was only slightly different than the alfalfa diet (25.4 vs. 24.0%) because 95% of the calcium in the alfalfa diet was from alfalfa. The true absorption of corn silage was significantly higher than alfalfa (52.0 vs. 25.8 %). The value of 52% compared

favorably with the value of 52% observed in our laboratory (unpublished) for corn silage fed to dry cows consuming about 85% the NRC required calcium for dry cows.

The partial true absorption of phosphorus (Table 4) was higher for alfalfa compared to corn silage (64.9 vs. 81.5%). We have observed a true absorption value for phosphorus from corn silage of 87% for dry cows. Our observed value of 65% for alfalfa is lower than the 81 to 96% reported by Lofgren and Kleiber, but agrees favorably with the values observed in our laboratory.

Table 1. Formulation of alfalfa (A) and alfalfa-corn silage (A-CS) rations fed to lactating cows (DM basis).

Ingredient	Percent DM		Calcium supplied		Phosphorus supplied	
	A	A-CS	A	A-CS	A	A-CS
Alfalfa	(%)	(%)	(g/d)	(g/d)	(g/d)	(g/d)
Alfalfa	33.7	24.1	103	74	29	21
Corn Silage	—	41.5	—	24	—	17
Corn Cobs	22.5	—	5	—	2	—
Hominy Grits	39.1	29.0	—	—	9	6
Corn Gluten Meal	3.9	3.9	1	1	4	4
Urea	.9	.9	—	—	—	—
Dynamate ^a	.2	.2	—	—	—	—
Salt, Trace Mineral ^b	.7	.7	—	—	—	—
Total			109	99	44	48

^bTrace mineral salt: Manganese 2,000, Iron 5,000, Zinc 2,000 ppm.

^aDynamate: Trademark by IMC, Inc. Mendilene, Illinois. Potassium 18, Magnesium 11, Sulfur 22 %.

Table 2. Dry matter intake and milk production of cows consuming alfalfa (A) and alfalfa-corn silage (A-CS) rations.

Ration	df	DM Intake	Apparent Digestion DM	FCM	Milk Fat	Milk Protein	Somatic Cells
A		(kg/d)	(%)	(kg/d)	(%)	(%)	(thou)
A		20.94	62.2	32.01	3.51	2.77	106
A-CS		22.34	74.5	35.21	3.35	2.70	110
SED		.47	1.84	1.70	.15	.13	40
Statistical Source							
Period	2	NS	NS	NS	NS	NS	NS
Animal	3	NS	NS	NS	NS	NS	NS
Diet	1	NS	P<.05	P<.10	NS	NS	NS
Residual	1	NS	NS	NS	NS	NS	NS
Error	5						

Table 3. Calcium and phosphorus balance and true absorption for cows consuming alfalfa (A) and alfalfa-corn silage (A-CS) rations.

RATION	df	Ca	Phos	Phos	Calcium	Endo.	True	Endo.	True
		Intake	Intake	Balance	Balance	Fecal Ca	Abs. Ca	Fecal Phos	Abs. Phos
.....(g/d).....									
A		104.1	31.5	-12.1	-36.7	16.9	24.0	5.8	64.2
A-CS		122.6	48.1	-5.0	-19.6	23.4	45.2	9.7	74.5
SED		4.6	1.57	1.15	3.3	7.9	4.7	3.0	6.7
Statistical Source									
Period	2	NS	NS	NS	P<.10	NS	NS	NS	NS
Animal	3	NS	NS	NS	NS	NS	NS	NS	NS
Diet	1	P<.05	P<.01	P<.01	P<.01	NS	P<.05	NS	NS
Resid.	1	NS	NS	NS	NS	NS	NS	NS	NS
Error	5								

Table 4. Partial true absorption for alfalfa and corn silage (CS) when fed to lactating dairy cows.

	Calcium				Phosphorus			
	True Absorption	Partial True Absorption						
Feed	(g/d)	(%)	(g/d)	(%)	(g/d)	(%)	(g/d)	(%)
Alfalfa Ration	25.4	24.0			20.4	64.2		
Alfalfa-CS	55.6	45.4			36.0	74.5		
Alfalfa			24.8	25.8 ^a			15.5	64.9 ^c
Corn Silage			17.0	52.0 ^b			26.4	81.5 ^d

^{a,b}Means differ P<.01 SED = 6.8

^{c,d}Means differ P<.05 SED = 7.1

IN VITRO DETERMINATION OF MINERAL SOLUBILITY FROM FORAGES

D.R.LEDOUX and F. A. MARTZ

Introduction

In order for dairy nutritionists to formulate diets that will provide the optimum requirements of the dairy cow it is essential that such diets be formulated based on available nutrients. Data on mineral availability from forages is limited due to the very high costs and time associated with *in vivo* studies. The development of an *in vitro* procedure to predict mineral availability from forages would be of considerable benefit to the dairy industry.

The objectives of this study were to: (1) determine the extent of mineral solubility from forages using *in vitro* techniques; (2) develop a pretreatment for forages that would simulate the ruminant digestive process; (3) determine the relationship (if any) between mineral solubility and true absorption (*in vivo*).

Materials and Methods

A randomized complete block design with a 6x6 factorial arrangement of treatments was used. Forages evaluated were alfalfa (2 samples; A1, A2), bluestem (BL), brome (B), corn silage (C), and fescue (F). In vitro treatments included: (1) 2X distilled water (W), (1) .01 N hydrochloric acid (H), (3) .172% pepsin solution (P), (4) .172% pepsin in .01 N hydrochloric acid (PH), (5) stage 1 of the "Tilley and Terry" procedure (T), (6) both stages of the "Tilley and Terry" procedure (TP; pepsin-HCl treatment reduced to 24 H). Three inorganic sources of Ca, limestone (L), calcium carbonate (CC), and calcium oxalate (CO) were included in the calcium solubility determination resulting in a 9x6 factorial arrangement. For treatments 1-4, 50 ml of each solvent was added to in vitro bottles containing .5 g of ground forage (1 mm screen) and the bottles capped and placed into a water bath at 39 C for 24 h. During the 24 h incubation bottles were swirled by hand 3 times. The same sample size (.5 g) was also used for treatments 5 and 6 with a buffer to rumen inoculum ratio of 4:1 (50 ml). Mineral solubility was determined from the ratio of mineral in total digest supernatant (samples centrifuged at 3000 rpm for 5 min) to that in the total mixture before incubation. Initial pH of solvents and final pH of digests were also determined. Forage samples were analyzed for NDF and ADF using the Van Soest procedure. Crude protein was determined by the Hach procedure. Calcium and Mg were analyzed by atomic absorption spectroscopy while P was determined by a colorimetric procedure. Data were analyzed by analysis of variance procedures by the GLM procedures of SAS. The model included replicate, forage, solvent and the interaction of forage and solvent.

Results and Discussion

Data on forage composition are presented in table 1. Forage samples exhibited a wide range for all variables measured.

In vitro mineral solubility and final pH of digests are presented in table 2. Of the forages, with the exception of the TP treatment, Ca from alfalfa was the least soluble. This was probably due to the fact that approximately one third of the total Ca in alfalfa is bound to oxalate and calcium oxalates are only solubilized at low pH. Calcium solubility from forages was similar for W and P treatments. Calcium solubility from the H and PH treatments were also similar but were higher than values from the W and P treatments. The lowest Ca solubility values were observed from the T treatment while the highest solubility occurred in the TP treatment. Of the inorganic sources only the TP treatment resulted in significant Ca solubility. As expected, CO was the least soluble (25 %) even at a final digest pH of 1.16. Calcium solubility was negatively correlated ($R=-.90$, $P<.001$) with final pH of digests, with higher Ca solubilities observed for treatments with lower pH.

Unlike Ca, P solubility was not related to pH. In general, P was more soluble than Ca, with solubilities ranging from 67 to 102% compared to a range of 18 to 108% for Ca. Phosphorus from C and F was substantially more soluble than from other forages and the effect was consistent across all treatments. This suggests that P from C and F may be more available. However, microbial digestion treatments (T and TP) resulted in P solubilities greater than 93%, indicating that once exposed to the rumen environment there was no longer a difference in solubility.

Similar to Ca, Mg solubility was found to be negatively correlated ($R=-.66$, $P<.001$) with final pH of digests although the relationship was not as strong. Of the forages, Mg from alfalfa was the least

soluble in treatments W, P, and T. Magnesium solubility from all forages was greater than 96% for treatments H, PH and TP.

With the exception of Alfalfa, mineral solubility values determined in this study suggests that solubility is not a limiting factor in the ruminant's ability to absorb these minerals since solubility values all exceeded in vivo true absorption values (Ca, 38%; P, 50%; Mg, 11-37%) cited by NRC (1988).

Table 1. Forage Composition

Forage	Ash	CP	NDF	ADF	Ca	Mg	P
%*							
Alfalfa-1	8.9	16.4	56.1	37.9	1.44	.17	.23
Alfalfa-2	8.3	19.1	47.7	31.7	1.32	.20	.30
Bluestem	4.9	10.0	74.2	37.6	.32	.19	.19
Brome	5.4	5.3	62.2	36.4	.53	.11	.11
Corn Silage	2.2	8.0	40.3	18.3	.27	.17	.22
Fescue	9.7	16.1	59.3	27.5	.52	.21	.31

*Dry matter basis

Table 2. In vitro mineral solubility and final pH of digest

Forage	W	H	P	PH	T	TP	Statistics		
							Effect	P	SE
<u>Ca, %</u>									
A1	33.3	79.0	33.0	76.2	18.1	107.0	Replicate	.0093	4.0
A2	37.8	84.7	42.8	82.0	18.3	108.3	Forage (F)	.0001	.7
BL	55.1	98.7	53.4	100.6	57.5	101.7	Solvent (S)	.0001	.8
B	60.4	102.1	62.7	102.6	44.5	105.2	F X S	.0001	2.0
C	69.6	100.8	73.8	101.8	77.0	101.8			
F	58.9	104.1	63.2	104.8	45.8	102.0			
L	.8	7.8	3.0	7.8	.6	74.8			
CC	.7	7.9	3.1	8.7	.7	81.6			
CO	.7	3.1	1.4	3.1	1.7	25.0			
<u>P, %</u>									
A1	78.6	76.2	83.3	87.3	93.2	99.5	Replicate	.0015	2.7
A2	76.8	77.0	86.9	86.5	94.8	99.6	Forage (F)	.0001	.6
BL	72.4	68.0	67.4	79.6	102.0	100.5	Solvent (S)	.0001	.6
B	72.7	64.8	71.4	86.2	100.0	101.2	F X S	.0001	1.4
C	96.7	93.1	99.5	95.2	102.7	100.5			
F	89.0	81.3	90.3	89.2	100.1	101.5			
<u>Mg, %</u>									
A1	68.3	96.9	67.0	97.7	80.9	98.4	Replicate	.0001	3.4
A2	70.9	102.1	77.7	101.8	82.1	97.9	Forage (F)	.0001	.7
BL	83.8	105.1	80.9	105.8	95.0	99.5	Solvent (S)	.0001	.7
B	80.3	101.0	83.5	101.5	94.7	99.5	F X S	.0001	1.7
C	92.7	107.5	97.6	95.2	99.9	102.9			
F	80.5	100.7	86.4	101.7	94.2	98.2			
<u>pH</u>									
A1	6.10	2.68	6.20	2.86	7.03	1.17	Replicate	.3581	.17
A2	5.95	2.60	5.23	2.86	7.08	1.13	Forage (F)	.0001	.03
BL	5.26	2.26	5.63	2.40	6.79	1.08	Solvent (S)	.0001	.03
B	4.52	2.23	4.42	2.35	6.82	1.08	F X S	.0001	.09
C	4.57	2.21	4.27	2.34	6.80	1.07			
F	5.23	2.40	5.31	2.67	6.96	1.11			

EFFECT OF DIET FIBER LEVEL AND FORAGE SOURCE ON INTAKE AND MILK PRODUCTION OF HOLSTEIN COWS IN MID OR LATE LACTATION

M.S. ALLEN and D.R. MERTENS

Introduction

Optional rations for dairy cows must contain adequate amounts of fiber as well as protein and energy. If too little fiber is fed, the rumen becomes too acidic and milk fat depression and intake reduction can occur. If too much fiber is fed, feed intake is limited by too much bulk in the ration and milk production declines. Previous research indicated that neutral detergent fiber (NDF) can be used to formulate rations that meet both the fiber and energy requirements of lactating cows. The NDF concept is based on the theory that cows maintain an equilibrium between ruminal fill and energy demand. Optional diets are formulated so that both fill and energy intake are maximized. The major objective was to measure ruminal characteristics of cows fed diets that met or exceeded their fiber or energy requirements. In addition, the experiment was designed to study the relationships between level of production, forage source and fiber concentration in the total ration. The production data will be presented in this report.

Materials and Methods

Three ration NDF levels were offered to nine high producing Holstein cows (<9000 kg mean milk/lactation) allocated to one of three forage sources: chopped alfalfa hay, alfalfa silage, corn silage, in a replicated 3X3 Latin Square (28 day periods) at two stages of lactation. A covariate period was used to allocate cows to forage and for adjustment of dependent variables. Total mixed rations of forage, high moisture corn and protein supplement at 25, 30 or 35% NDF in stage 1 of lactation (avg 160 days in milk) or 30, 35 or 40% NDF in stage 2 of lactation (avg 250 days in milk) were offered once a day at a 10% refusal level. Rations were balanced for protein and minerals.

Results and Discussion

Mean milk production was 30.9 kg for stage 1 and 18.6 kg for stage of lactation. Forage source had no effect on milk fat amount or fat corrected milk at either stage; however, in stage 1 milk production and dry matter intake as a percent of body weight were higher and milk fat % was lower for alfalfa hay than either silage (figure 1). NDF level was significantly ($P<.01$) related to 3.5% fat corrected milk, milk fat percent, and milk fat amount for stage 1 only (figure 2). Total milk production was less affected ($p<.08$) and dry matter intake as a percent of body weight was not affected by NDF level for either stage of lactation. Optimum NDF% for fat corrected milk was 32.8 in stage 1 of lactation. These values were similar for dry matter intake as a percent of body weight. The optimal acid detergent fiber (ADF) concentration in the total ration was more variable than for NDF (figure 3). In stage 1, the optimal ADF was approximately 16% for corn silage compared to 21% for alfalfa. As with NDF, an optimal ADF for stage 2 was not definable with only three cows per treatment. Concentrations of NDF in the total ration was inversely related to milk protein percentage in both stages of lactation (figure 4). The milk protein content of cows fed alfalfa silage was consistently lowest for all NDF concentrations while cows fed alfalfa hay produced milk with the highest milk protein percentage.

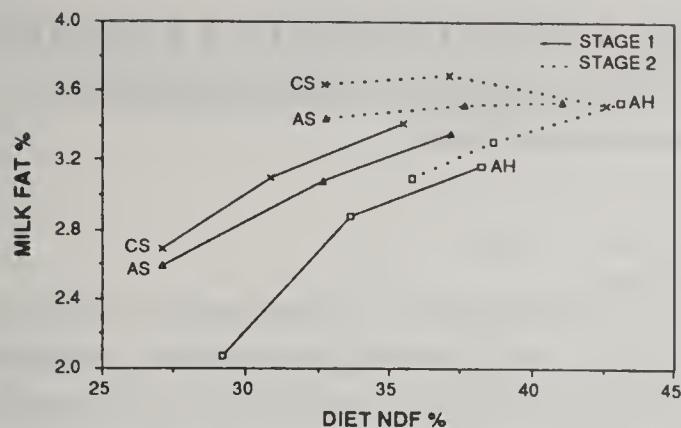


Figure 1. Relationship of milk fat percentage at two stages of lactation to dietary neutral detergent fiber (NDF) when alfalfa hay (AH), alfalfa silage (AS) or corn silage (CS) were the forage sources.

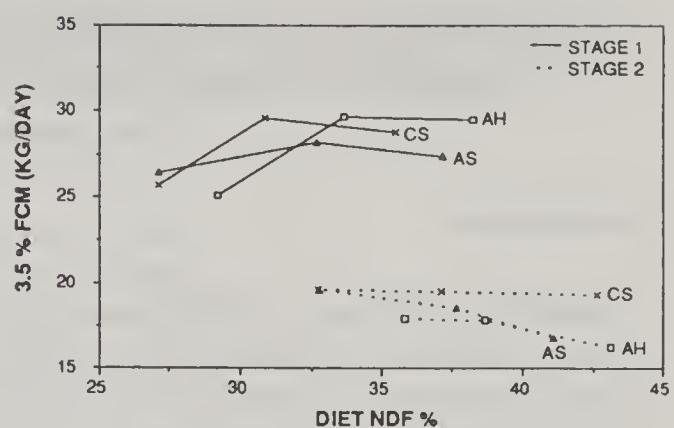


Figure 2. Relationship of milk production in two stages of lactation to dietary neutral detergent fiber (NDF) concentration when rations contained alfalfa hay (AH), alfalfa silage (AS) or corn silage (CS) as the forage sources.

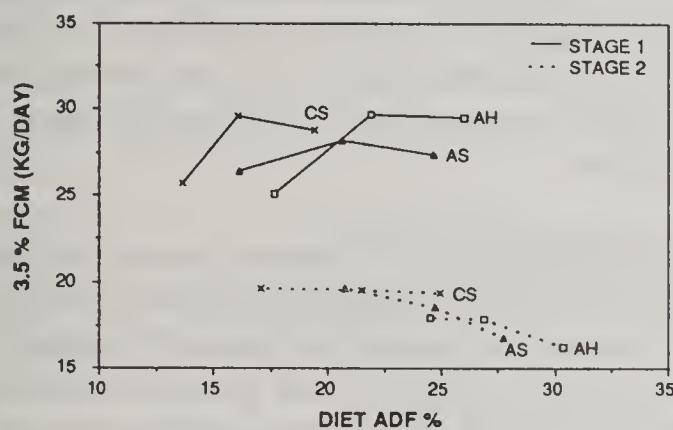


Figure 3. Response of 3.5% fat corrected milk at two stages of lactation to acid detergent fiber (ADF) content of the ration when alfalfa hay (AH), alfalfa silage (AS) or corn silage (CS) were the forage sources.

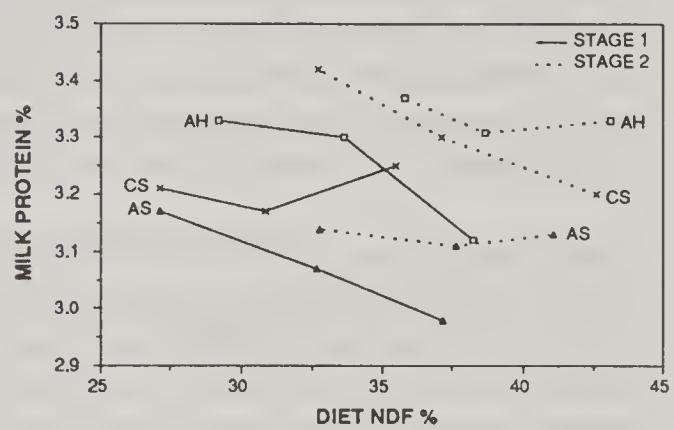


Figure 4. Responses of milk production at two stages of lactation to neutral detergent fiber (NDF) content of rations containing alfalfa hay (AH), alfalfa silage (AS) or corn silage (CS).

MEAN RETENTION TIME IN SHEEP AS INFLUENCED BY FORAGE PARTICLE LENGTH AND MORPHOLOGICAL COMPONENTS

D.J. CHERNEY and D.R. MERTENS

Introduction

Production by ruminants on high forage diets is often limited by intake. Retention time of dry matter in the rumen is one of the main factors controlling intake. Improvement in procedures to estimate mean retention time of forages should lead to improvements in intake predictions. It was hypothesized that differences in forage particle length and rates of passage of morphological components are primary factors affecting retention time of matter in the rumen. Our objective was to determine the influence of forage particle length and morphological component on mean retention time of particles in the gastro-intestinal tract.

Materials and Methods

In each of two periods 24 sheep, aged 12-18 months, were offered one of 12 hays (4 animals/hay) at:1) 100% of what was eaten in an ad libitum period (L1) and 2) 1.8% of body weight (L2). Forages offered included sorghum-sudan (2 hays), barley (4 hays), oat (4 hays) and pearl millet (2 hays) hays. Hays were similar in neutral detergent fiber (NDF) concentrations (613 ± 25 g NDF eaten kg^{-1} dry matter), but varied in morphological composition.

Mean retention time of ND extracted large (2.5 cm) and small (ground to pass a 1 mm screen) stems (ST), and large and small leaf blades (LB) were determined using europium (Eu), lanthanum (La), ytterbium (Yb) and cerium (Ce), respectively. An immersion technique was used to fix markers to particles. Rare earths were added to particles at a rate of 20 ug g^{-1} fiber. Four grams of each marked particle fraction were dosed simultaneously to each sheep at each level of forage offered. Fecal samples were collected 17 times (0 to 144 h) post dosing for each level of forage offered.

Three compartment models of passage were developed and solved for marker fecal excretion. Models were fitted using iteratively reweighted nonlinear least squares with weight related to the inverse of the residuals. These models were then used to obtain mean retention time of the markers.

Results and Discussion

As intake decreased from L1 to L2, mean retention time of morphological components increased (Table 1). Retention times of ST were greater than those of corresponding LB in L1 and L2. Large ST had longer mean retention times than small ST in L1 and L2 (56.9 vs 47.2 h and 66.3 vs 54.5 h for L1 and L2, respectively). Large LB had a longer mean retention time than small LB in L1 (45.2 vs 41.8 h), while no differences were observed in L2 (51.0 h). It is concluded that level of feed intake influences the mean retention time of large and small particles, regardless of their source.

Within each hay, LB particles were retained for a shorter time than ST particles (Table 2). In general, large ST had the largest mean retention times and small LB had the smallest. Small LB varied little between hays while the mean retention times of large ST was inversely related to the proportion of LB in the forage. We conclude that length and morphology of marked particles influences measured mean retention time. Small particles (LB and ST) may be associated with fluid rather than

particle flow and may not adequately reflect retention times of the whole forage being fed. Determining the particle size distribution and mean retention time of both large and small particles may lead to improvements in intake predictions.

Table 1. Mean retention time of marked particles at two levels of intake.

Marker	Marker	Intake Level 1 ^a	Intake Level 2 ^b
		(h)	(h)
Eu	Large stems	56.9	66.3
La	Small stems	47.2	54.5
Yb	Large leaf	45.2	51.0
Ce	Small leaf	41.8	51.0
	BLSD ^c	1.75	2.16

^aDaily intake was 2.1% of body weight.

^bDaily intake was 1.7% of body weight.

^cBayes least significant difference
(K=100, P approximately .05).

Table 2. Mean retention times for marked fractions of individual hays.

Species	Hay	%NDF	% Leaf	Marker ¹			
				Eu	La	Yb	Ce
Sorghum- sudan	1	59.9	85.2	48.2	— ²	39.9	43.8
	2	60.5	88.1	49.5	— ²	57.3	46.1
Oats	3	62.0	15.1	62.0	52.8	44.3	46.2
	4	64.1	12.0	67.8	53.5	51.6	48.7
	5	65.4	8.8	73.7	53.4	52.9	47.1
	6	61.7	11.5	58.7	44.0	46.0	45.4
Barley	7	59.9	3.2	61.5	51.8	44.4	41.1
	8	60.9	13.2	65.1	49.2	43.5	43.5
	9	62.2	7.6	70.1	52.0	45.7	45.4
	10	60.7	3.6	63.5	51.0	48.0	47.0
Pearl	11	59.6	84.8	53.6	42.9	53.3	41.6
Millet	12	59.1	70.5	48.7	46.4	48.3	44.3
	BLSD ^c			6.17	6.33	7.58	8.00

^aBayes least significant difference (k=100, P approximately .05).

¹Eu=europium, marked large ST particles; La=lanthanum, marked small ST particles; Yb=ytterbium, marked large LB particles; Ce=cerium, marked small LB particles.

²Not enough marked ST for these hays.

EVALUATING MATURITY AND PRESERVATION METHOD OF ALFALFA ON OPTIMUM RATIONS FOR LACTATING DAIRY CATTLE BASED UPON NEUTRAL DETERGENT FIBER CONCENTRATION OF THE TOTAL DIET

R.W. HINTZ, D.R. MERTENS and K.A. ALBRECHT

Introduction

Research has shown that formulating rations based upon neutral detergent fiber (NDF) concentration of the total diet can be an effective means of maximizing the performance of lactating dairy cattle. Results of these studies suggest that differences in the optimum NDF level may exist between forages preserved in different manners. To test the hypothesis that the method of forage preservation affects the optimum NDF concentration of diets, a study was designed to compare the response of lactating dairy cattle to diets of identical NDF concentration formulated using third-crop alfalfa forages of two maturities (mid-bud and late flower) that were preserved as hay, low-moisture silage, and high-moisture silage.

Materials and Methods

Six forages (Table 1) were used to formulate total mixed rations containing 20.5, 25, 29.5, or 34% NDF. All diets were formulated to contain a minimum of 17% crude protein, 0.90% calcium, 0.48% phosphorus, 0.24% magnesium, and 0.46% salt on a dry matter basis. The 24 resulting diets were fed to 48 lactating Holstein cows (12 primiparous and 36 multiparous) in a series of six replicated 4 X 4 Latin squares. Each Latin square consisted of a single forage type with the four NDF levels within a forage type constituting treatments within each square. A standardization diet of 29.5% NDF was fed to all animals prior to beginning the experiment and was used as a covariate adjustment.

Results and Discussion

The animals assigned to diets containing the early maturity forages exhibited higher milk production and DMI during the standardization period than animals assigned to diets containing late maturity forages. Thus the genetic potential of animals is confounded with maturities and makes direct comparisons between forage maturities subject to bias. However, trends in the data and relative relationships can be compared.

For both maturities there were no significant differences among preservation methods for production of 4.0% SCM or for the NDF concentration of the diet that maximized DMI and production of 4.0% SCM. Both maturities exhibited milk fat depression for diets containing less than 29.5% NDF and a decrease in protein concentration of the milk was noted for diets containing more than 25.0% NDF. Non-corrected milk production was greatest when diets contained 20.5% NDF for both maturities of alfalfa.

Preservation method did not affect ($P > 0.05$) DMI for diets containing early maturity alfalfa, but DMI was greater for high-moisture silage than hay or low-moisture silage for diets composed of late maturity alfalfa (Table 2). DMI and production of 4.0% SCM were maximized by NDF concentrations of 24% for the early maturity forage and 26% for the late maturity forage. Some straw bedding

may have been consumed by cows fed the rations with low NDF concentrations, resulting in observed optimum NDF concentrations lower than the actual optimum NDF consumed. The NDF concentration that maximized performance was relatively constant across forages, even though substantial differences in the forage to concentrate ratio of diets were observed. The forage to concentrate ratio at the optimum NDF concentrations ranged from 39:61 for hay diets containing late maturity forage to 49:51 for high-moisture silage diets containing early maturity forage. The results of this study indicate that preservation method and possibly forage maturity do not alter the effectiveness of using NDF to formulate rations and substantiates previous reports that NDF is a useful and reliable criteria for formulating diets for lactating dairy cows.

Table 1. Chemical composition of forages used in this study.

		Preservation				
Maturity	Method*	%DM	%CP	%NDF	%ADF	%ADL
Early	Hay	89.0	20.7	44.7	30.1	6.42
	LMS	47.3	21.8	39.8	28.1	6.10
	LMS	31.7	22.3	37.4	27.2	6.17
Late	Hay	83.9	19.6	51.0	32.9	8.22
	LMS	46.0	20.8	45.9	30.2	7.32
	HMS	31.8	20.2	45.7	29.9	7.30

*LMS=Low-moisture silage, HMS=High-moisture silage.

Dry matter for hay samples determined by 105° C oven, dry matter for silage samples determined by toluene distillation.

Crude protein=nitrogen X 6.25.

Table 2. Effect of preservation method of alfalfa on the performance of lactating dairy cattle, averaged over dietary NDF concentrations and adjusted for differences in covariate performance.

PRES	DMI (kg/d)	MILK (kg/d)	FAT (%)	PROTEIN (%)	LACTOSE (%)	4.0% SCM (kg/d)	F:C RATIO**
Diets Containing Early Maturity Alfalfa							
HAY	23.4	33.9	3.45	3.03	4.84	30.9	39:61
LMS*	22.6	32.0	3.46	3.06	4.77	29.0	45:55
HMS*	22.3	33.8	3.37	2.98	4.80	30.0	49:51
LSD (P=0.05)	3.0	5.2	0.63	0.24	0.24	3.3	
Diets Containing Late Maturing Alfalfa							
HAY	21.4	31.4	3.25	3.13	4.80	27.6	39:61
LMS*	24.0	31.4	3.47	3.18	4.80	28.8	44:56
HMS*	26.3	31.8	3.24	3.18	4.79	28.5	44:56
LSD (P=0.05)	2.8	4.5	1.03	0.13	0.14	3.7	

*LMS = Low moisture silage, HMS = High moisture silage.

**Forage to concentrate ratio at the estimated optimum NDF concentration.

FORAGE SOURCE AND INTAKE LEVEL ON DRY MATTER DIGESTIBILITY

R. S. CARDOZA and D.R. MERTENS

Introduction

Forage evaluation involves both in vivo and chemical characterization. Animal performance is the ultimate measure of forage quality, but chemical characterization of forage quality is needed for routine forage evaluation and ration formulation. Differences among forages in intrinsic plant characteristics may provide information needed to identify and understand plant factors limiting digestion and intake. However, experimental design and animal factors, such as genetic potential, physiological state and intake, can also influence animal performance and the assessment of forage quality. The purpose of this research project was to identify and measure the indigestible residue in forages and determine the relationships of plant and animal factors to forage digestibility.

Materials and Methods

Forty-eight sheep (30-60 kg) were used to measure intake and digestibility of twelve forages in two experimental periods. A randomized complete block design was used with sheep assigned to treatment by weight and breed. Each experimental period consisted of three feeding levels: (1) ad libitum (8-12% refusal rate), (2) ad libitum (0% refusal rate) and (3) maintenance (1.8% body weight). Feed offered, feed refused and feces weight were recorded daily. Total fecal collection began 48 hours after the first feeding of the collection period at each intake level to allow for transit through the gastrointestinal tract. Dry matter (DM) was determined by drying in a convection oven at 105°C for 24 hours. Neutral detergent fiber (NDF), acid detergent fiber (ADFs) sequentially extracted from NDF and 72% sulfuric acid lignin (SLIG) sequentially extracted from ADFs were analyzed by modifications of the Goering and Van Soest methods. Forages were selected to provide a diversity of species and lignin concentration. Legumes were represented by ladino clover (LC), red clover (RC) and birdsfoot trefoil (BFT). Birdsfoot trefoil was selected because of its reported high tannin content. Cool-season perennial grasses were represented by bromegrass (BRO), orchardgrass (ORG) and timothy (TIM). Within each species two hays were selected to obtain the widest range of SLIG concentration in the NDF (Table 1).

Results and Discussion

A wide range of forage quality is indicated by the variation in lignin concentration in both DM and NDF (Table 1). Legumes had 2-4 times greater SLIG:NDF than grasses. However, legumes had greater dry matter digestibility (DMD) and dry matter intake (DMI) than grasses. Within forage species, DMD decreased with increasing SLIG:NDF except for ORG (Table 1). Orchardgrass yielded similar DMD and SLIG with different NDF whereas BFT had different DMD with similar NDF and SLIG. Inconsistencies in the relationships between composition and DMD indicate that these forages may be useful in identifying additional factors affecting digestibility. Dry matter digestibility increased with decreased feeding level (Table 1). Slower rates of passage out of the rumen when DMI decreased was the probable cause of the increased DMD.

Both NDF and ADFs are correlated ($P<0.01$) with DMD (Table 2). However, analyzing by species type indicates only legume DMD is correlated ($P<0.01$) with NDF, ADFs and SLIG whereas grass DMD is correlated ($P<0.01$) only with SLIG (Table 2). The poor correlations in grasses are partially

related to the small variation in DMD of this group. Legume DMI was correlated ($P<0.05$) with NDF, ADFs and SLIG but not in grasses (Table 2). With all forages the positive correlations of SLIG and SLIG:NDF with DMD and DMI indicate an interaction between forage types and suggests that relationships are valid only within a forage type. Differences among and between legume and grass results indicate further characterization is needed to ascertain which components are limiting DMD and DMI.

Table 1. Effect of forage source and fiber composition on dry matter intake (DMI) and dry matter digestibility (DMD).

FORAGE ¹	NDF ² (%)	ADFs ² (%)	SLIG ² (%)	INTAKE LEVEL ³				DM DIGESTIBILITY			
				1	2	3	MEAN (%BW)	1	2	3	MEAN (%)
BFT	45.6	34.1	8.75	2.8	2.6	1.6	2.3	62.3 ^a	61.3 ^a	62.1 ^a	61.9 ^f
BFT	43.3	33.1	8.24	3.3	2.9	1.6	2.6	65.4 ^a	65.5 ^a	65.2 ^a	65.4 ^e
LC	33.2	23.1	5.30	3.6	3.4	1.6	2.9	67.7 ^a	68.2 ^a	71.2 ^b	69.0 ^c
LC	30.8	21.6	3.76	3.2	3.0	1.6	2.6	66.3 ^a	67.5 ^a	70.3 ^a	68.0 ^d
RC	54.2	39.7	7.66	2.2	2.1	1.7	2.0	56.8 ^a	57.5 ^a	56.7 ^a	57.0 ^h
RC	39.8	28.6	4.54	3.2	2.9	1.6	2.6	67.2 ^a	67.5 ^a	70.1 ^b	68.3 ^{cd}
BRO	68.9	40.0	4.64	2.0	1.7	1.6	1.8	56.6 ^a	53.9 ^b	56.2 ^a	55.6 ⁱ
BRO	68.4	38.1	3.90	2.6	2.2	1.7	2.2	56.3 ^a	56.0 ^a	59.7 ^b	57.4 ^h
ORG	63.3	35.8	2.94	2.3	2.1	1.6	2.0	56.6 ^a	55.8 ^a	57.7 ^b	56.7 ^h
ORG	56.9	30.8	2.43	2.7	2.5	1.8	2.3	56.7 ^a	56.1 ^a	59.1 ^b	57.3 ^h
TIM	66.6	36.0	4.05	2.2	2.0	1.6	1.9	55.6 ^a	57.2 ^b	57.3 ^b	56.7 ^h
TIM	63.8	35.6	2.90	2.1	1.9	1.6	1.9	63.4 ^a	64.4 ^a	65.9 ^b	64.6 ^e
Mean				2.7	2.5	1.6		60.9 ^a	60.9 ^a	62.6 ^b	

¹ Forages: BFT = birdsfoot trefoil, LC = ladino clover, RC = red clover, BRO = bromegrass, ORG = orchardgrass and TIM = timothy

² Analysis of bale core samples for neutral detergent fiber (NDF), sequential acid detergent fiber (ADFs) and sequential 72% sulfuric acid lignin (SLIG)

³ Intake level: (1) ad libitum (8-12% refusal rate), (2) ad libitum (0% refusal rate) and (3) maintenance (1.8% body weight)

^{a,b} Means within a row with different superscripts differ ($P<0.05$)

^{c,d,e,f,h,i} Means within a column with different superscripts differ ($P<0.05$)

Table 2. Correlations of fiber components with maintenance (feeding level 3) dry matter digestibility (DMD) and ad libitum (feeding level 1) dry matter intake (DMI).

VARIABLE FORAGE TYPE:	DMD			DMI		
	ALL	LEGUME	GRASS	ALL	LEGUME	GRASS
NDF	-0.79**	-0.91**	-0.20	-0.76**	-0.73**	-0.25
ADF	-0.81**	-0.91**	-0.21	-0.72**	-0.70**	-0.31
LIGNIN	0.02	-0.75**	-0.44**	0.21	-0.42*	-0.28
LIGNIN:NDF	0.42**	-0.33	-0.49**	0.54**	0.01	-0.28
LIGNIN:ADF	0.41**	-0.26	-0.49**	0.54**	0.07	-0.24
DMI (%BW)	0.01**	-0.60**	0.02			

Significance levels: *($P<0.05$) and **($P<0.01$)

A MODEL OF FORAGE UTILIZATION AND ANIMAL PERFORMANCE

D.R. BUCKMASTER, C.A. ROTZ, D.R. MERTENS and J.R. BLACK

Introduction

A model of the dairy forage system (DAFOSYM) is used to evaluate and compare technologies and management strategies for the dairy farm. An important part of the total system is the feed utilization and milk production performance of the animals on the farm. The original version of DAFOSYM included a rather crude model of feed disappearance with no attempt to predict animal performance for a given ration. Development of a new model was undertaken to create a more complete model which 1) allocated feeds to a dairy herd in an efficient manner and 2) predicted feed intake and milk production for animals fed the allocated ration.

Materials and Methods

A model of animal intake and performance was developed which represented the 'state-of-the-art' in dairy nutrition. The primary source of information for the model was the latest edition of "Nutrient Requirements of Dairy Cattle" by the National Research Council. The model consisted of two major parts: feed allocation and animal performance. Farm produced feeds were allocated to the dairy herd according to animal requirements and feed availability. Possible feeds included: 1) low-quality alfalfa (hay or silage), 2) high quality alfalfa (hay or silage), 3) corn silage, 4) high-moisture ground ear corn, and 5) dry corn grain. When required, these feeds were supplemented with purchased feeds of 1) soybean meal, 2) distillers grains, 3) corn grain, and 4) medium quality hay.

For feed allocation the herd was split into 6 groups and a feeding order was strategically chosen to allocate feeds where they were best used by the animal. Dry cows were fed first, heifers greater than one year of age were fed second and heifers under one year old were third with a mix of low quality alfalfa and corn silage as the preferred forage. Feed requirements for these groups were determined first so that if there was a shortage of forage, the higher quality hay purchased would be fed to lactating cows. The remaining three groups were lactating cows at 3 stages of lactation. Highest producers were fed first and lowest producers last. The preferred forage was a mix of high quality alfalfa and corn silage.

The preferred forage mix for any group was used when available; if not, an alternative forage mix was used. When high quality alfalfa was preferred, low quality alfalfa was the alternative, and vice versa. If both low and high quality alfalfa stocks were depleted, purchased medium quality hay was used. The ratio of hay to alfalfa silage and/or total alfalfa to corn silage in the forage mix was determined from the amount left in storage. The preferred corn in the ration was always high-moisture ground ear corn. The first alternative was corn grain from storage, and the second was purchased corn grain.

To model animal performance, rations were formulated for each of the 6 groups of animals such that 5 criteria were satisfied. If the feeds available could not provide the necessary nutrients for the given production level (yet satisfy intake and fiber limitations), the production level was decreased to that level which could be supported with the given feeds. Criteria for meeting dairy cow feed requirements were: 1) animals have a limited capacity to ingest fiber, 2) 75% of daily neutral detergent fiber intake should come from forages, 3) energy requirement must be met, 4) ammonia pool in the

rumen must be adequate for microbial growth, and 5) substrate must be available in the rumen for microbial growth. The rations were determined by implementing a linear programming algorithm used as an inequality problem solver. The "objective" function was to maximize forage use while using as little purchased energy and protein supplement as possible.

Energy and crude protein requirements were calculated as documented by the National Research Council. Degradability of crude protein in hay and silage vary substantially, which results in differences in utilization of protein by lactating cows. In order to more clearly assess the impact of forage preservation on lactation performance and ration supplementation, the absorbed protein system was used. Several modifications to this system were made to improve agreement with crude protein requirements and protein digestibility and to insure that reasonable rations were formulated for use in DAFOSYM. These modifications included: 1) separating endogenous fecal losses from total absorbed nitrogen, 2) increasing the true digestibility of available rumen escape protein to 95%, 3) subtracting acid detergent insoluble protein from total escape protein to obtain available escape protein, and 4) calculating degradability of alfalfa silages as a function of protein solubility.

Results and Discussion

The animal model was incorporated into DAFOSYM where it was used to evaluate technology in forage production. A formal validation of this type of model was not possible. The model accuracy was verified by comparing feed requirements predicted by the model to those suggested by the National Research Council. Feed disappearance simulated by the model was very reasonable for typical dairy herds. The new model increased the sensitivity of farm performance to forage quality, especially for high producing herds. Technology which improves the quality of forage now shows a greater economic return when analyzed with the latest version of DAFOSYM as compared to older versions. Much of the increase in return occurs because the new model allows maximum milk production to increase as forage quality improves.

RUMINAL PEPTIDES: A PREVIOUSLY UNRECOGNIZED NITROGEN POOL

J. B. RUSSELL

Introduction

For many years nutritionists have sought ways of reducing the wasteful degradation of dietary protein in the rumen. In many cases, solubility was correlated with the degradability of protein, and by the 1970's it was generally assumed that solubilization was the rate limiting step in protein degradation. Inverse relationships between protein solubility and nitrogen retention cannot be ignored, but recent studies indicated that peptides accumulated in vitro and in vivo.

Materials and Methods

Mixed rumen bacteria or pure cultures were incubated in vitro with casein or pancreatic digests of casein containing small peptides. Hydrophilic and hydrophobic peptides were isolated from pan-

atic digests by isopropyl alcohol (90%) precipitation. Ammonia was measured by the colorimetic method of Chaney and Marbach (1963). In vivo experiments were conducted with non-lactating cows or first lactation cows fitted with ruminal canulae using a 3 x 3 Latin square design. Diets contained corn silage, hay crop silage, barley, corn meal, minerals, and various combinations of soybean meal (SBM), extruded soybean (ESB) or fish meal. The rations were fed 12 times per day or once a day. Ruminal dilution rate was estimated from the decrease in Co-EDTA. Peptides were measured by a procedure involving perchloric acid precipitation, the removal of ammonia under alkaline conditions, and the assay of amino acids after HCl hydrolysis.

Results

When mixed rumen bacteria were incubated in vitro with growth rate limiting amounts of mixed carbohydrates and an excess of casein, casein hydrolysis was accompanied by a marked accumulation of peptides which were not metabolized rapidly by the rumen bacteria. Rumen fluid from a cow fed timothy hay and concentrate supplement (16% crude protein) contained more than 1.2 g peptides per liter, 1 h after feeding, and this value declined to the prefeeding value of 0.4 g/liter by 8 h after feeding. Before feeding the average peptide size was approximately 8 amino acids, but this value declined to approximately 4 soon after feeding.

When rations supplemented with SBM were fed 12 times per day and crude protein (CP) was 14.5, 17.1 or 20.6%, the estimated flow of peptides from the rumen was 138, 206 and 206 g/day, respectively. When the soybean meal was autoclaved to decrease protein solubility (14.4, 16.9, and 19.3 % CP) the flow of peptide was 119, 163 and 194 g/day, respectively. In both these experiments rumen volume and dilution rate remained relatively constant throughout the day and flow was proportional to concentration. If the cows were fed rations containing 17.8% CP once a day, the peptide concentration and dilution rate was highest 2 hours after feeding and declined significantly thereafter. Replacement of half the soybean protein with either ESB or FM decreased the flow of peptides from the rumen, but the flow was still greater than 88 g/day.

Hydrophobic peptides which dissolved in 90% isopropyl alcohol contained an abundance of phenolic and aliphatic amino acids, while the hydrophilic peptides which were precipitated by alcohol contained a large proportion of highly charged amino acids. Mixed rumen bacteria degraded hydrophilic peptides more than twice as fast as hydrophobic peptides.

Discussion

Peptide accumulations were originally observed in cultures containing casein, but in vivo results indicated that these accumulations were not an artifact of casein hydrolysis. Peptide flow from the rumen was as great as 200 g per day, an amount which would be sufficient to make 25% of the casein in 50 pounds of milk. The identification of peptide utilization as another rate limiting step, suggests that amino acid composition and protein solubility may not be the only criteria affecting protein degradation by ruminal bacteria. Since hydrophobic peptides were utilized more slowly than hydrophilic peptides, the degradation of a particular amino acid (eg. lysine) could be affected by which amino acids are adjacent to it in the protein.

THE IMPACT OF ALFALFA MATURITY AND PRESERVATION METHOD ON MILK PRODUCTION OF COWS IN EARLY LACTATION

W.F. NELSON and L.D. SATTER

Introduction

This experiment is the third in a series of lactation trials we have conducted to assess the impact of maturity and preservation method on the value of alfalfa as a feed for dairy cows. It is commonly believed that milk production decreases with increasing maturity of alfalfa fed. This relationship is not well quantified and may be sensitive to many variables including energy demand, fraction of forage in the diet, method of preservation of the forage, season of harvest and climatic conditions during plant growth. In two previous studies mid lactation cows were used, and it was observed that maturity had little effect on milk production for cows producing up to 27 kg milk/day with silage being less sensitive to an increase in maturity than hay. This experiment was designed to study the effects of preservation method and maturity on the milk production of cows during the first ten weeks of lactation.

Methods and Procedures

Two maturities of second cutting alfalfa were harvested in 1987. Half of each maturity was preserved as silage in bag silos, and half was preserved as dry hay. Table 1 contains morphological and chemical profiles of the four forages harvested. Sixteen primiparous and 32 multiparous Holstein cows were assigned to one of the four forage treatments on day 15 of lactation subsequent to a two week covariate period. The covariate diet contained 30% early cut silage (ECS), 30% late cut silage (LCS) and 40% grain mix (dry basis). Each of the four treatment diets contained 60% alfalfa [ECS, LCS, early cut hay (ECH) or (LCH)] and 40% grain mix on a dry basis. Silage diets were fed as total mixed rations once daily. Cows on hay diets received hay once daily in hanging hay feeders placed in the manger, while grain was fed twice daily. All diets were formulated to provide 19% CP. Milk production and feed intake were measured daily. Body weights were taken and milk samples were analyzed for fat and protein weekly.

Results and Conclusions

Milk production values for weeks four through ten of lactation were adjusted for the covariate period and are shown along with body weight (BW) data in Table 2. Intake and milk composition parameters are not adjusted for the covariate period. Increasing alfalfa maturity had no significant effect on milk production or dry matter intake. However, across both maturities cows fed alfalfa silage diets produced more milk (2.3 kg/d) and consumed more dry matter (1.5 kg/d) than cows consuming hay diets. Neither forage maturity nor method of preservation had a major impact on milk composition. Increasing forage maturity did not appear to detrimentally influence BW changes, but silage diets provided for greater gains of BW than hay diets.

The results of this study, combined with two earlier trials, suggest that the impact of increasing alfalfa maturity on the intake and production of dairy cattle is considerably less than previously estimated. In three lactation trials, comprising three years, two cutting seasons and 188 cows at various stages of lactation and ranging in production from 16.0 kg/d to 35 kg/d, the greatest impact

of alfalfa maturity on milk production observed was a decrease of .15 kg/d each day cutting was delayed past the prebloom (bud) stage of maturity.

The apparent advantage of feeding alfalfa silage relative to alfalfa hay in this trial may be due, in part, to management factors inherent in feeding silage and hay. Worthy of further investigation is the possibility that there are factors which favor forage fiber utilization in silage, resulting in increased intake and production when compared with hay of similar quality.

Table 1. Forage maturity and composition.

	Morphological Stage	Date cut	Mean stage by weight*	%CP	%NDF	%ADF	%ADL
Early cut silage	Midbud	6/26/87	3.35	21.1	37.9	31.0	6.24
Late cut silage	Midflower	7/9/87	5.50	18.1	45.3	35.3	8.03
Early cut hay	Midbud	6/26/87	3.35	19.1	44.9	32.7	6.81
Late cut hay	Midflower	7/9/87	5.50	17.9	48.8	36.6	8.57

*Kalu and Fick, 1981.

Table 2. Response of early lactation cows to four alfalfa forages fed as 60% of the ration dry matter. (Values are means of observations obtained from week 4 through 10 of the treatment period.)

	Actual Milk kg/d	Adjusted Milk kg/d	Milk Fat %	Milk Prot. %	DMI kg/d	DMI % of BW	NDFI kg/d	NDFI % of BW	BW Change kg/d
Early cut silage	33.2	33.5	3.42	2.74	19.8	3.40	11.9	.92	-.04
Late cut silage	31.2	33.1	3.32	2.70	18.4	3.51	12.4	1.07	+.10
Early cut hay	30.4	30.7	3.37	2.69	17.5	3.40	11.5	1.01	-.50
Late cut hay	30.4	31.3	3.44	2.65	17.7	3.30	12.3	1.04	-.23
p values,*									
EC vs LC	.47	NS	NS	.41	.38	NS	.13	.03	ND
Silage vs hay	.21	.03	NS	.30	.02	.42	NS	.42	ND

*NS = not significant, greater than .50; ND = not determined

OPTIMIZING HEAT TREATMENT OF FULL FAT SOYBEANS TO INCREASE PROTEIN AVAILABILITY FOR THE RUMINANT

M.A. FALDET, L.D. SATTER, G.A. BRODERICK, and D.B. RICKER

Introduction

Ruminants that have high protein requirements, such as dairy cattle in early lactation, are dependent upon a large supply of dietary protein which escapes microbial degradation in the rumen but is

available for digestion in the small intestine. For this reason, various methods of feed processing or treatment have been examined to increase the resistance of protein to ruminal degradation. It is important, however, that the treatment leading to protein protection in the rumen does not reduce digestibility of protein in the small intestine. Heat treatment appears to have the greatest potential for safe and economical treatment of protein. Though results have been positive for feeding heat protected proteins in some studies, there is no evidence in the literature of efforts to optimize heat treatment for specific protein supplements. Therefore, the objective of this study was to determine the relationship among protein degradation in the rumen, protein availability in the small intestine and heat input (dependent on temperature and time duration) in order to determine the optimal point of heat treatment for full fat soybeans.

Material and Methods

Full fat soybeans were heated in a forced-air oven at different temperatures for different lengths of time. Two types of measurements were used to determine optimum heat treatment of soybeans. One was to measure the degree of protection that different amounts of heat will give against microbial protein degradation in the rumen. An in vitro inoculum containing inhibitors of nitrogen uptake by the microbes was used to estimate rate of ruminal protein degradation. The second type of measurement was to determine the availability in the intestine of amino acids from heat treated soybeans. Since lysine is the most vulnerable amino acid in terms of heat damage and is considered to be one of the limiting amino acids for milk production in dairy cows, its nutritional availability was measured.

Two methods were used to measure nutritionally available lysine (NAL). One was an indirect 1-fluoro-2, 4 dinitrobenzene method (total lysine minus inaccessible, TLMI) and the other was a rat growth (RG) assay.

Rumen undegradable protein (UDP) was estimated using in vitro protein degradation rates (IVDR) which were corrected for acid detergent insoluble nitrogen, and assuming a rumen passage rate of .06/h. The product of UDP and NAL (using results from RG or TLMI) was used to estimate the amount of lysine which escapes the rumen and is available for intestinal absorption.

Results and Discussion

In general, as heat input increased, estimated UDP increased while IVDR and NAL decreased (See table). As temperature increased, the time required to maximize estimated post-ruminal NAL decreased. A loss of 15-20% of NAL when using TLMI values was necessary to achieve the greatest increase in estimated flow of NAL to the small intestine. Even though both estimates of NAL did not exactly agree, it did not impair detection of the point of optimal heat treatment. The greatest deviation of the two methods for determining NAL was with the soybeans that had a low amount of heat input (i.e. 140°C-10 min). This was apparently due to the anti-growth factors (i.e. trypsin inhibitor) in the soybean samples which would influence rat growth and therefore reflect a lower NAL value. The apparent optimum heat treatment for soybeans appears to be at 140°C>120 min, 150°C-60 min and 160°C-30 min using a forced air oven.

Table 1. Effect of Roasting Temperature and Time on Lysine Supply to the Ruminant

Temp °C	Duration Minute	IVDR /h	UDP %	Nutritionally Available Lysine		Post-Ruminal Nutritionally Available Lysine	
				TLMI g/100g SB	RG g/100g SB	TLMI g/kg SB	RG g/kg SB
0	0	.152	29.7	2.43	1.98	7.22	5.88
100	60	.108	36.7	2.27	1.99	8.33	7.30
	180	.101	38.7	2.21	1.89	8.56	7.31
130	60	.101	38.2	2.36	1.95	9.02	7.45
	180	.066	48.0	2.14	1.74	10.3	8.35
140	10	.124	33.9	2.44	2.06	8.37	6.98
	30	.078	43.9	2.20	2.09	9.66	9.18
	60	.061	49.4	2.17	1.97	10.7	9.73
	90	.048	55.0	2.01	1.89	11.1	10.4
	120*	.043	59.2	1.89	1.81	11.2*	10.7*
150	10	.108	36.6	2.39	2.08	8.75	7.61
	30	.080	42.4	2.19	2.07	9.29	8.78
	60*	.041	58.4	1.99	1.85	11.6*	10.8*
	90	.032	64.2	1.56	1.50	10.0	9.6
	120	.025	69.9	1.56	1.46	10.9	10.2
160	10	.104	37.4	2.33	2.10	8.71	7.85
	30*	.050	53.2	2.07	2.09	11.0*	11.1*
	60	.022	72.0	1.41	1.35	10.2	9.7
	90	.024	71.1	1.14	1.08	8.11	7.68
	120	.019	75.1	1.06	.99	7.96	7.43

*Optimal Treatment

IVDR - In vitro degradation rate

UDP - Rumen undegradable protein

TLMI - Total lysine minus inaccesible

RG - Rat growth assay

RESPONSE TO POSTRUMINAL INFUSION OF PROTEIN DURING EARLY LACTATION IN DAIRY COWS FED ALFALFA SILAGE

T.R. DHIMAN and L.D. SATTER

Introduction

The energy and protein requirement of the lactating dairy cow in early lactation is known to be very high. Cows fed diets with a high proportion of alfalfa silage seem to be protein deficient due to high rumen degradability of protein in alfalfa silage. Rumen by-pass of protein might improve the performance of lactating dairy cows fed with alfalfa silage based diets. The objective of this experiment was to study the effect of post ruminal infusion of protein in early lactation cows fed with all alfalfa silage diets.

Material and Methods

Twelve multiparous Holstein cows were randomly assigned to three treatments (4 cows in each treatment) at parturition. An adaptation period of 14 days was given during which all the cows were

fed with diet 1 (forage:grain, 48.2:50). On day 15 of lactation cows in treatment 1 were fed with diet 1 and cows in treatment 2 and 3 with diet 2. In addition to this, cows in treatment 3 were infused with 1 kg. casein (1185 g Ca-caseinate) into the abomasum through the rumen cannula. The ingredient and chemical composition of diets 1 and 2 are given in Table 1. The experimental period lasted until cows were 12 weeks in lactation.

Daily feed intake and milk yield were measured. A twice weekly composite of A.M. and P.M. milk samples were analyzed for milk composition. Body weights were recorded once a week. Blood samples were taken once a week from tail vein/artery and were analyzed for blood glucose and plasma β -hydroxybutyrate.

Results and Discussion

Responses to experimental diets are shown in Table 2. Milk yield, protein yield and dry matter intake were significantly ($P<0.05$) higher in treatment 1 as compared to 2 and 3. Milk fat % was not affected by different treatments but daily fat yield was lower with the all forage diet because of lower milk yield. Milk protein % and protein yield were significantly increased ($P<0.04$) due to casein infusion as compared to the all forage diet. There was a 22% increase in fat corrected milk yield due to post ruminal infusion of 1 kg. casein per day when all alfalfa silage diet was fed. In treatment 3, infusion of casein did not change the daily dry matter intake.

During the experimental period cows in all the treatments lost bodyweight as cows were in early lactation. Cows fed with only alfalfa silage in treatment 2 lost more weight as compared to the other two treatments. Because of high variability among animals the differences were not significant.

Average values for blood glucose are shown in Fig. 1. Blood glucose concentration was higher in treatment 1 followed by treatment 3 and 2. Cows infused with casein had higher blood glucose concentration than cows fed with the same diet without casein infusion, which suggests that part of the casein was used as a glucogenic source.

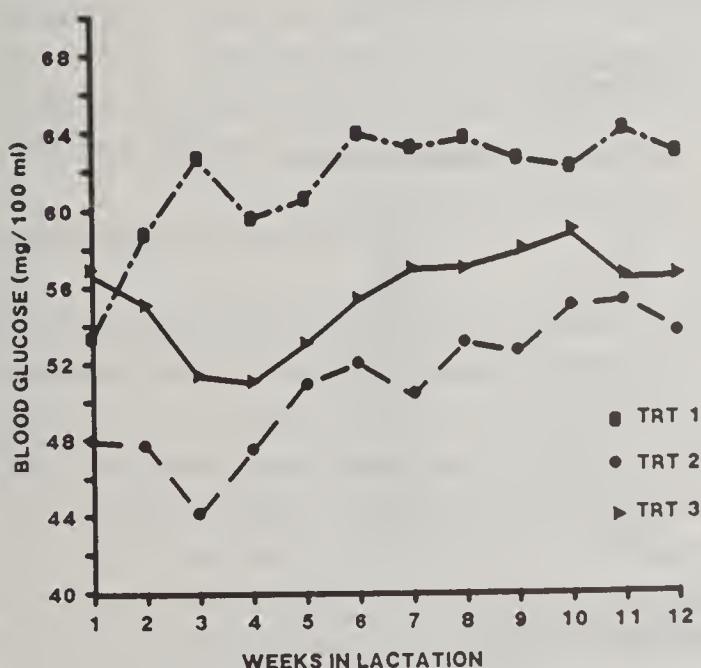


Figure 1. Treatment effect on blood glucose.

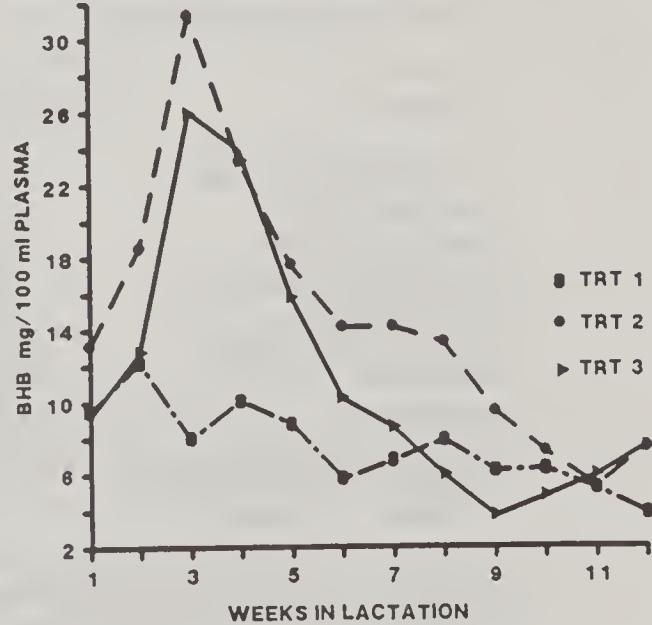


Figure 2. Treatment effect on β -hydroxybutyrate in plasma.

During week 2 to 6 of lactation cows in treatment 2 and 3 had a higher concentration of plasma β -hydroxybutyrate (Fig 2). This decreased as the lactation advanced. A high concentration of β -hydroxybutyrate indicates that cows were using body reserves to meet their energy demand during early lactation.

The increase in milk and milk protein yield due to casein infusion suggests that high alfalfa silage diets were not able to supply enough protein in the small intestine and rumen by-pass protein supply may improve the performance of the cows in early lactation.

Table 1. Ingredient composition of experimental diets (% dry matter basis)

Ingredient	Diet 1	Diet 2
Alfalfa silage	48.2	98.2
High moisture ear corn	34.3	—
Soybean meal	15.7	—
Dicalcium phosphate	1.1	1.1
Trace mineralized salt	0.7	0.7
Additional Vit. A, D, E		
<u>Chemical Composition</u>		
	CP %	NDF %
DIET 1	18.3	27.9
DIET 2	16.4	41.1
		ADF %
		19.4
		33.2

Table 2. Treatment effect on milk yield, milk composition, DM intake and body weight change.

Parameter	Treatment		
	1 (F:G, 48.2:50)	2 (All Forages)	3 (Forage+Casein)
Actual Milk Yield, Kg/d	46.1	29.7	35.8
Milk yield, Kg/d ¹	44.5 ^a	30.7 ^b	36.2 ^c
3.5% FCM, Kg/d ^{1,2}	41.6 ^a	31.3 ^b	38.2 ^a
Actual Milk Protein %	2.88	2.63	2.78
Milk Protein % ¹	2.85 ^a	2.66 ^b	2.77 ^{ab}
Milk NPN, mg/100 ml ^{1,3}	32.3	30.9	30.6
Actual Milk Fat %	3.40	3.78	3.38
Milk Fat % ¹	3.25	3.62	3.68
Dry matter intake, Kg/d	25.1 ^a	18.4 ^b	19.6 ^b
Total body weight change, Kg (Week 12 - Week 2)	-11.1	-32.3	-13.2

^{abc}Means in the same row with different superscript differ (P<.05)

¹Means have been adjusted for covariate.

²3.5% FCM = [.432*(Kg Milk)] + [16.2*(Kg Fat)]

³Non-Protein Nitrogen

MEASURING HYDRATION KINETIC AND WATER HOLDING CAPACITY OF FORAGES WITH PYCNOMETERS

M.A. WATTIAUX, D.R. MERTENS and L.D. SATTER

Introduction

Hydration of feedstuffs may be an important factor influencing feed consumption by ruminants. In vitro studies have demonstrated that fiber sources holding more water are fermented to a greater extent. Not only the amount of water held, but also the kinetics of water uptake might be important in microbial attachment, digestion kinetics associated with lag time, and changes in specific gravity of forage particles in the rumen. Even though many techniques are available to measure water holding capacity, none gives a kinetic insight to the hydration process. Therefore, a technique was developed to measure the parameters of hydration kinetics of forages.

Materials and Methods

In vitro digestion solutions and rumen fluid inoculum were prepared. They were mixed, filtered through glass wool and autoclaved. Sodium azide (0.5 g/l) and Penicillin G (10^5 units/l) were added to the sterile solution to prevent microbial growth.

The equivalent of 2 g DM of 2 mm ground Alfalfa Hay (AH), alfalfa silage (AS), and bromegrass hay (BH) samples were placed into pycnometers. Forty ml of sterile solution were added, and the samples were allowed to hydrate under vigorous stirring. Every 10 min. for the first two hours, 15 min. for the third hour, and 30 min. for the forth and fifth hours, solution was added to adjust the total volume, and the weight of the pycnometer was recorded. All the measurements, were performed after solution and samples had equilibrated in a warm room at 39°C.

The residual (insoluble) DM was then recovered by filtration through a double layer of 53 μm pore size dacron filter (Centrifugation can also be used), and dried at 105°C. The experiment was designed as a randomized complete block with 3 replicates.

The uptake of solution (expressed as g/g residual DM) over time was fitted to a one or two pool exponential models to determine the fractional rate(s) of hydration and the total uptake of solution. A "mean accumulation time" was calculated to estimate the time required to accumulate half the total of solution uptake.

Results and Discussion

Because the specific gravity of the solution was nearly 1.00, the weight added at each time interval represents the hydration (g of water uptake) as well as the volume of pore space (ml of gas) displaced during that interval. The intercept of the curve represents the total water uptake (Fig. 1). The kinetics of uptake were best described with 1 and 2 pool models for the silage and the hays, respectively. Table 1 shows that AH accumulated about 16 times more solution at a rate 3 times faster than AS. BH accumulated less solution but at the same rate as AH. The standard error of the means ($n=3$) of the kinetic parameters indicates good repeatability of the technique. The water holding capacity of the residual DM calculated by adding the initial moisture content to the total solution uptake (g water/g residual DM) was: 1.94, 1.42, and 1.18 for AH, AS, and BH, respectively.

This technique permits the measurement not only of the water holding capacity but also the kinetic of water accumulation that might have a physiological significance during the processes of ruminal digestion and passage of forage particles.

Table 1. Hydration characteristics of alfalfa hay, alfalfa silage, and bromegrass hay incubated in autoclaved rumen fluid and buffer solution.

Forage	Alfalfa Silage	Alfalfa Hay	Bromegrass Hay
Uptake of Water (g/g residual DM)	0.12 ^a (0.01)	1.81 ^b (0.04)*	1.08 ^c (0.01)
Mean Accumulation Time (min.)	60.1 ^b (10.1)	20.2 ^a (3.3)	23.0 ^a (2.4)

^{a,b,c}: Means with different superscripts are different (P<.05).

*: Standard error of the means, n=3.

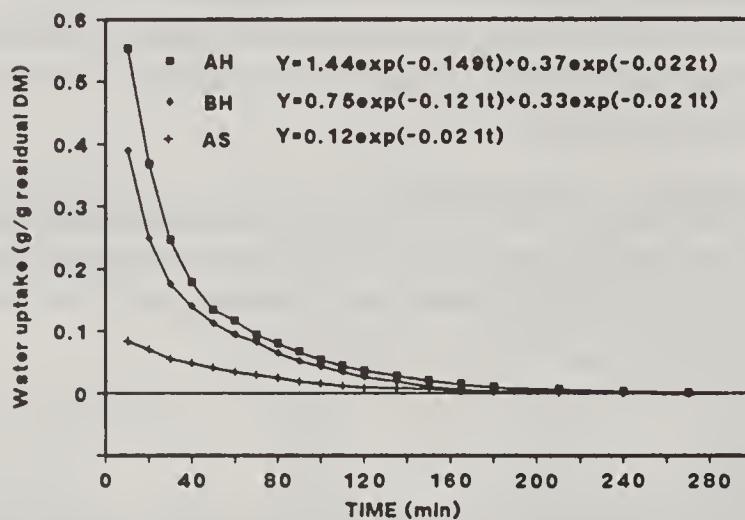


Figure 1. Uptake of water by two g DM of alfalfa hay (AH), alfalfa silage (AS), and bromegrass hay (BH) incubated in autoclaved buffer and rumen fluid.

EFFECT OF MICROBIAL FERMENTATION ON SPECIFIC GRAVITY OF ALFALFA HAY INCUBATED IN VITRO

M.A. WATTIAUX, L.D. SATTER and D.R. MERTENS

Introduction

Specific gravity (SG) may be an important characteristic influencing rumen retention time of forage particles. Previous work has indicated an increase in SG with incubation time in the rumen. It has been hypothesized that partially digested particles tend to be buoyant because of gas entrapment. As fermentation proceeds, gas production decreases, the buoyancy effect decreases and consequently the SG of the residue increases. This experiment was designed to test the buoyancy hypothesis. The change in Functional SG (FSG) of alfalfa hay (AH) during in vitro digestion was measured and related to changes in gas production.

Materials and Methods

Rumen fluid inoculum was prepared and enriched with particulate associated microorganisms. The equivalent of 1.5 g DM of AH ground through a 2 mm screen were placed into pycnometers and mixed with 42 ml of inoculum. Volume measurements were made every 3 hours for the first 30 hours of incubation. A mixture of distilled water, 2-ethoxyethanol (ethylene glycol monoethyl ether), and 0.1N NaOH in the ratio 8:2:1 was used as displacing solution to adjust the total volume of the pycnometer before the total weight was recorded.

The DM and fluid fractions were then separated by centrifugation (5500 g * for 25 min). The DM recovery was determined after oven drying at 55 °C. The SG of the fluid fraction at 39 °C was measured by weighing 10 ml of solution. FSG of the residual DM was calculated for each incubation time as described by Hooper and Welch (1986). The gas production from the pycnometer was allowed to displace a pH 5.3 buffered solution from a 25 ml measuring pipette. The experiment was replicated once with three observations per incubation time. Protected LSD was used to compare FSG at each incubation time.

Results and Discussion

The weights and the volumes of the residual DM are presented in figure 1. Functional volume is an estimate of the volume of both DM and gas associated with it. For the first nine hours of incubation, even though DM disappears, the functional volume (ml/g residual DM) increases. Swelling of the fiber and/or increase in gas volume are possible explanations. During the period of 12 to 30 hours of incubation, the functional volume decreases more rapidly than the weight, suggesting that the gas fraction escapes from the residual digesta.

FSG is the inverse of functional volume adjusted for the SG of the incubation solution. Figure 2 shows that FSG of the digesta significantly increases over time ($P<0.05$) and is negatively related to the change in gas production ($r=0.66$, $P<0.01$).

The results of this study support the concept that feed particles are nucleation sites for gas bubble formation. During fermentation the pattern of change in both functional volume of the digesta and gas production are positively correlated ($r=0.63$, $P<0.02$) (Fig. 1 and Fig. 2). When the digesta

losses its buoyancy, FSG increases. If a critical value of 1.2 has to be reached before digesta is eligible for passage out of the rumen, the rate at which FSG increases might have a profound effect on digesta retention time in the rumen. In this study, AH would not be eligible for passage from the reticulo-rumen before 18 hours of digestion.

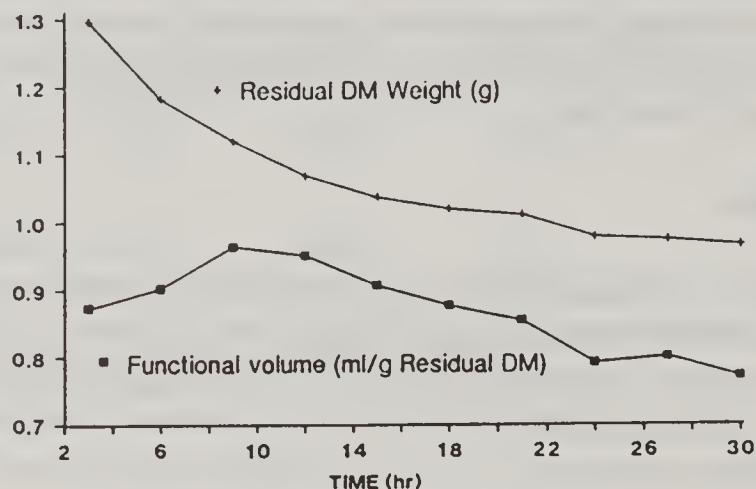


Figure 1. Change in weight and functional volume (volume of DM+gas associated with it) of 1.5g alfalfa hay during in vitro digestion.

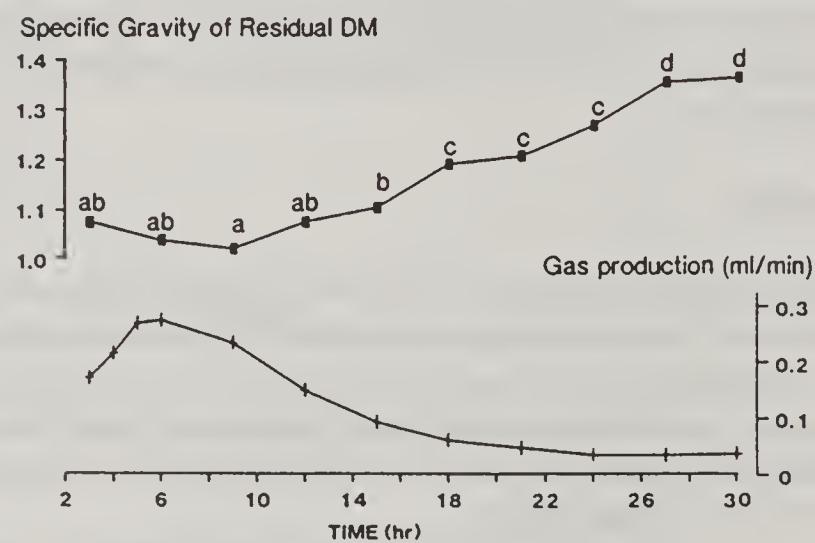


Figure 2. Specific gravity of residual DM and gas production during in vitro digestion of 1.5g alfalfa hay. Specific gravities with different letters differ ($p<0.5$).

ENERGY VERSUS PROTEIN SUPPLEMENTATION OF HIGH ALFALFA SILAGE DIETS FOR EARLY LACTATION COWS

C. CADORNIGA and L.D. SATTER

Introduction

Earlier studies have shown a marked depression in milk protein when diets with large amounts of alfalfa are fed. The reason for this decrease is attributed to high degradability of alfalfa protein. Better utilization of forage protein could be achieved by including in the diet rapidly fermentable carbohydrates to support microbial growth and synthesis of microbial protein. Similar results could be obtained by supplementing rumen undegradable protein. (RUP).

An experiment with early lactation cows was conducted to compare the effects of energy versus RUP supplementation of alfalfa silage based diets.

Materials and Methods

Thirty-nine animals (13 heifers and 26 mature cows) were assigned to four different treatments. The experiment included a 2 week covariate period following parturition when animals were fed diet 1, and a subsequent 12 week experimental period when they were fed one of four diets (1 to 4). Diets 1 and 4 served as positive and negative controls respectively; the test diets (2 and 3) were isoenergetic, but different in protein content (table 1).

Rations were fed once daily as a total mixed ration. Samples of rations and feed refusals were collected daily and composited weekly. Individual feed intake and milk weights were recorded daily; body weight was recorded weekly. Milk samples for fat and protein analysis were taken from four consecutive milkings and composited weekly.

Results and Discussion

Milk yield and composition, dry matter intake and average body weight are given in table 2. All values are least square means based on covariate period adjustments.

Diets 2, 3 and 4, according to NRC standards, are deficient in energy and adequate in crude protein. Diets 2 and 3, equal in energy content, differ in their ability to support milk production. Diet 3, containing the expeller soybean meal (a good source of undegradable protein), supported milk production almost equal to the high energy diet containing much more grain (table 2 and figure 1). Body weight change is shown in figure 2. This experiment supports the view that diets containing large amounts of high quality alfalfa, usually considered adequate in protein but deficient in energy, are actually deficient in protein (presumably due to high degradability of the protein in alfalfa) and perhaps better than previously thought in terms of supplying energy.

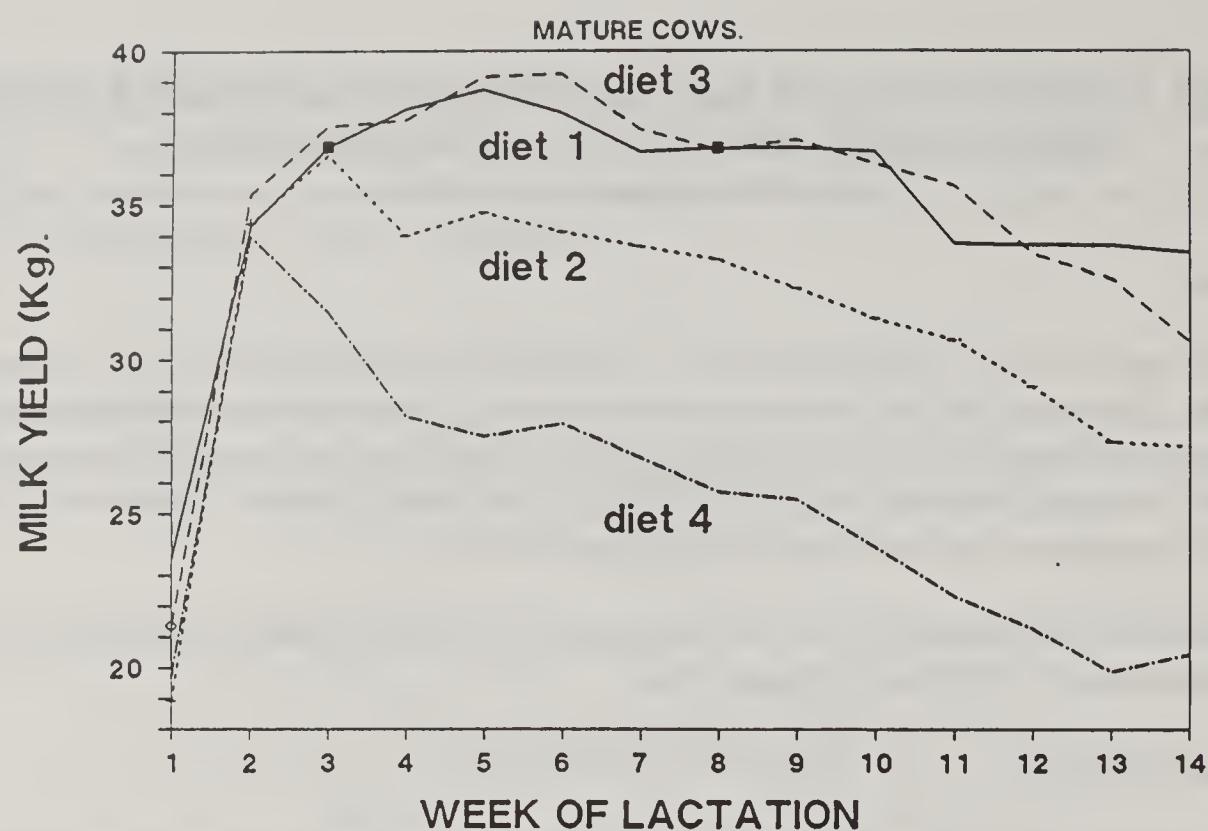


Figure 1. Average milk yield.

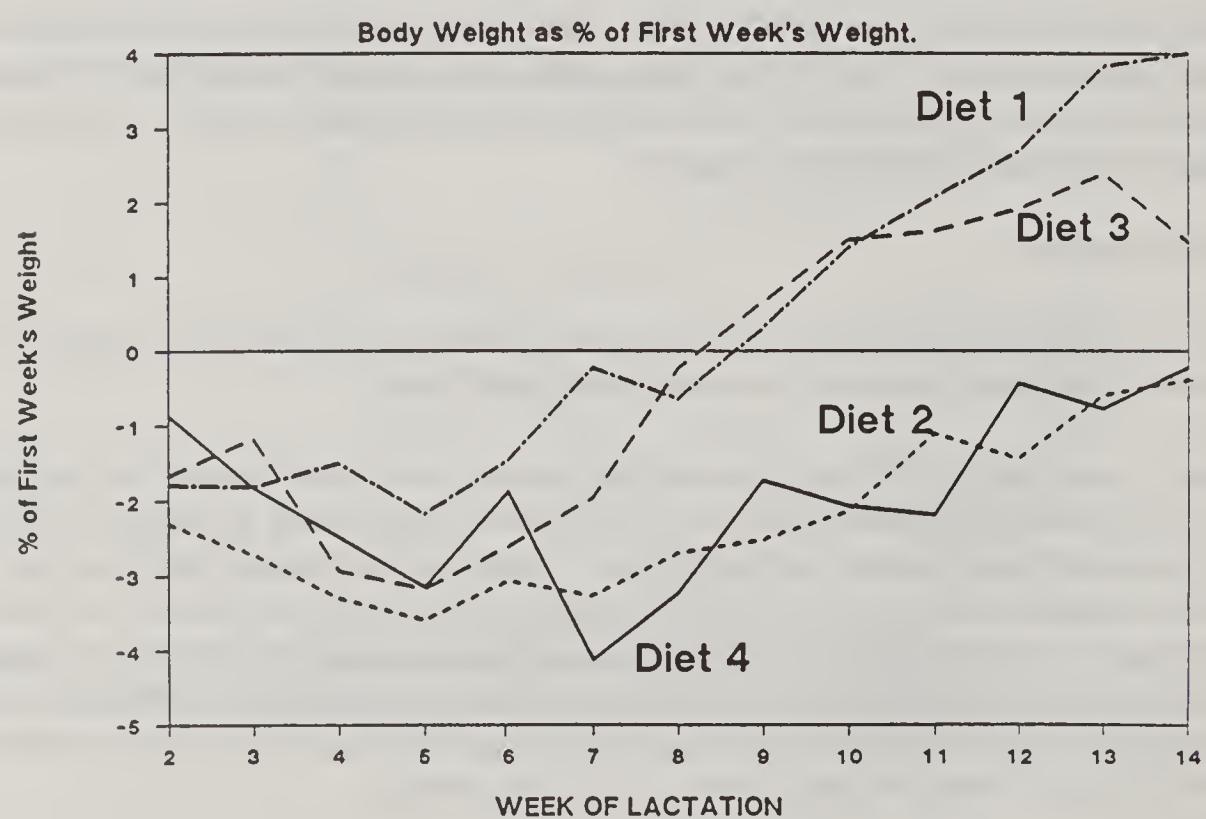


Figure 2. Body weight change.

Table 1. Diet Composition

	Diet 1	Diet 2	Diet 3	Diet 4
<u>Ingredients</u>	Dry Matter Basis			
Alfalfa Silage	48.2	75.0	75.0	98.2
High Moisture Ear Corn	40.0	23.2	—	—
Solvent Soybean Meal	10.0	—	23.2	—
Expeller Soybean Meal	—	—	—	—
Mineral-Vitamin Suppl.	1.8	1.8	1.8	1.8
<u>Composition</u>				
Crude Protein (%)	17.9	17.4	26.3	19.9
NE lactation (Mcal/Kg)	1.65	1.52	1.53	1.41

Table 2. Lactation performance (Mature cows only).

Measurement	Diets			
	1	2	3	4
Milk (kg/d)	36.3 ^d	32.1 ^f	35.5 ^e	25.5 ^g
Milk Protein (%)	2.93 ^a	2.77 ^{ab}	2.75 ^{ab}	2.63 ^b
Milk Fat (%)	3.54	3.58	3.42	3.57
Dry Matter				
Intake (Kg/d)	23.3 ^a	20.9 ^b	22.5 ^{ab}	18.8 ^c
Dry Matter				
Intake (% BW)	3.90 ^a	3.65 ^b	3.78 ^{ab}	3.43 ^c
Body Weight				
Change (Kg/d)	0.48 ^a	0.19 ^{ab}	0.25 ^{ab}	-0.09 ^b

^{a,b,c} : values in the same row differ (P<.05)^{d,e,f,g}: values in the same row differ (P<.1)

EFFECT OF MONENSIN ON PLASMA AMINO ACID CONCENTRATIONS IN HOLSTEIN HEIFERS FED HIGH MOISTURE ALFALFA SILAGE

J.M. LOPEZ-GUISA and L.D.SATTER

Introduction

Monensin, an ionophore produced by *Streptomyces cinammonensis*, has been widely used in ruminant nutrition to improve animal performance. Feeding monensin has generally increased feed efficiency with high grain diets and increased both feed efficiency and growth rate with high roughage diets. The mode of action of monensin has not been totally developed. However several changes in the rumen and animal have been observed and associated with a possible mode of action. Some of these changes are: reduction in the acetate: propionate ratio in the rumen, reduction of deamination and/or proteolysis in the rumen, lower concentration of ammonia in rumen fluid, increased blood-urea, increase in cellulolytic bacteria and higher nitrogen retention. Most of the studies reporting on the use of monensin have been with animals fed either high grain or low quality roughage diets. Diets with high moisture alfalfa silage containing a large amount of highly

degradable protein have not been reported. The addition of monensin to these diets could reduce proteolysis and/or deamination, and be reflected in increased concentration of essential amino acids, especially the branched chain amino acids. The objective of this study was to determine if monensin, fed with high alfalfa diets, significantly alters plasma concentrations of essential amino acids.

Materials and Methods

Forty Holstein heifers weighing 150 to 200 kg were divided according to weight, into heavy and light groups, and subsequently randomly assigned to the control or monensin diets. The control diet on a dry matter basis was 99.3 % alfalfa silage, 0.5 % trace mineral salts, 0.2 % monosodium phosphate, and a vitamin ADE supplement. Monensin was added to give 33 g monensin/kg. of feed (33 ppm). The animals were fed once daily, allowing a refusal of 5 %. Feed and feed refusals were taken daily and composited weekly. The length of the experiment was 11 weeks and followed a 3 week adaptation period to monensin. The heifers were weighed once a weekly except at the beginning and end of the experiment when they were weighed on three consecutive days. On day 69 feed was removed four hours prior to feeding to encourage prompt consumption of the feed. Blood samples were taken from the jugular vein three hours after feeding. Blood plasma was deproteinized and prepared for analyses. Urea and glucose in deproteinized plasma were determined.

Results and Discussion

No differences were observed in body weight gains between control and monensin groups. Feed intake was significantly lower for animals fed the monensin diet, and this resulted in improved feed efficiency. Plasma urea was similar for both treatments, and glucose was significantly lower for animals fed monensin. Plasma amino acids were measured as an indicator of the absorption of amino acids by the small intestine. Total and essential amino acids were significantly higher for animals fed monensin, indicating greater uptake of amino acids from the small intestine. Monensin may have a role in improving the protein status of ruminants fed diets containing large amounts of forage.

Table 1. Body weight gain, feed intake and feed efficiency, plasma urea, glucose and amino acids

	CONTROL	MONENSIN	MSE ^a
Weight gain (g/day)	979	947	209
Dry matter intake (kg/day)	6.98 ^b	6.59 ^c	099
NDF intake (kg)	2.67 ^b	2.50 ^c	033
Feed efficiency (kg feed/kg gain)	7.13 ^b	6.96 ^c	
Urea (mM)	6.54	6.76	116
Glucose (mg/100ml)	82.7 ^b	77.1 ^c	5.22
Plasma amino acids	mM		
Total	1542 ^b	1892 ^c	504
Essential	795 ^b	1041 ^c	232
Non-essential	747	851	285
Branched chain	361	410	110
Branched chain glycine	1.24	1.14	.116

a Mean square error.

b,c Means with different superscripts in the same row differ significantly (P<.05).

INFLUENCE OF A COMBINATION OF FEED INGREDIENTS ON PERFORMANCE OF DAIRY COWS IN EARLY LACTATION

R.G. DADO and D.R. MERTENS

Introduction

To promote maximal production in a previous experiment using fistulated cows, a dietary supplement containing optimal-to-excessive amounts of most factors thought or known to stimulate milk production was fed to the cows in early lactation. The supplement was formulated to contain high quality, rumen undegradable protein, added fat, slowly digestible non-fibrous carbohydrates, buffers, and extra vitamins, minerals, and branched chained fatty acids. The supplement appeared to stimulate production with 9 of 11 cows producing in excess of 45 kg of milk per day. The current study was conducted to statistically test the hypothesis that the dietary supplement would stimulate production of cows in early lactation.

Materials and Methods

Forty-eight multiparous and sixteen primiparous cows were assigned to one of two dietary treatments at parturition and remained on the study until 73 ± 20 days. Cows were blocked according to milk production levels in the previous lactation and freshening date. The control (C) and experimental (E) diets were formulated to contain 20% crude protein (CP) and 22.5% neutral detergent fiber (NDF) from alfalfa silage. Concentrate for diet C contained 72% high moisture ear corn (HMEC), 25% soybean meal, 2% dicalcium phosphate (dical), and 1% trace mineral salt (TMS) on a dry matter basis. Concentrate for diet E contained 38.7% HMEC, 16.9% ground shelled corn, 15.7% dried distillers grain, 15.3% heated and rolled soybeans, 3.87% dehydrated molasses, 2.69% Isoplus, 1.94% fishmeal, 1.45% meat scraps, 1.22% dical, 0.73% TMS, 0.67% zinc methionine, 0.60% sodium bicarbonate, 0.18% magnesium oxide, and 0.04% niacin on an as-fed basis. Rations were adjusted weekly to account for nutrient variability. Mixed diets were fed once daily in amounts to allow for 5-10% refusal. C and E diets contained 52.1 and 53.6% dry matter, 20.3 and 20.1% CP, 30.6 and 31.7% NDF, 21.4 and 21.4% acid detergent fiber, 4.2 and 5.9% ether extract, and 9.3 and 10.0% ash respectively.

Results and Discussion

Non-significant increases in milk production, percent fat, and percent protein were obtained over the twelve week experimental period with treatment E (Table 1). Both peak milk production and dry matter intake (DMI) occurred within the first 7 to 9 weeks of lactation with no significant differences in peak production between treatments. Peak levels of milk were high for both cows and heifers averaging 43 and 32 kg of milk respectively. NDF intake as a percent of body weight (NDFI%BW) was significantly higher for E heifers across the entire experimental period but not within any single week. Average NDFI%BW for the control treatment was 1.04% for heifers and 1.22% for cows. NDFI%BW was slightly higher for treatment E which contained by-product feeds. Milk production, DMI, and NDFI%BW increased linearly across weeks for both heifers and cows with no contrast differences between treatments. Body weight, body condition scores, and crude protein intake were not different between treatments; however, ruminal pH was higher for E animals.

Table 1. Production and intake by week of lactation and experimental period.

		Week of Lactation						
Trt		1	3	5	7	9	11	Average
Heifers ^b C	Milk (kg)	14.45	27.89	28.52	30.11	26.42 ^a	25.47	27.53
	%Fat	4.20	3.42	3.42	3.14	3.37	3.05	3.38
	%Protein	3.50	2.91	2.76	2.78	2.90	2.95	2.89
	DMI (kg) ^d	11.88	14.58	16.97	18.29	17.09	16.18	16.46
	NDFI%BW ^e	.75	.92	1.14	1.18	1.06	1.02	1.04 ^a
Heifers ^b E	Milk (kg)	14.05	29.26	31.26	33.40	32.76 ^a	29.50	29.88
	%Fat	4.81	3.70	3.57	3.44	3.22	3.26	3.59
	%Ptn	3.76	2.98	2.83	2.77	2.82	2.66	2.94
	DMI (kg) ^d	11.91	15.32	17.80	18.89	18.01	18.25	17.23
	NDFI%BW ^e	.93	.97	1.20	1.26	1.15	1.16	1.12 ^a
Cows ^c C	Milk (kg)	24.65	40.50	42.70	42.37	40.33	36.48	39.62
	%Fat	4.16	3.72	3.34 ^a	3.31	3.57 ^a	3.32	3.51
	%Ptn	3.58	3.06	2.79	2.86	2.90 ^a	2.97 ^a	2.98
	DMI (kg) ^d	16.83	20.60	23.58	25.15 ^a	25.56	24.09	23.17
	NDFI%BW ^e	.90	1.10	1.26	1.34	1.33	1.24	1.22
Cows ^c E	Milk (kg)	22.71	39.76	42.97	42.01	41.64	39.67	39.65
	%Fat	4.47	4.03	3.57 ^a	3.49	3.29 ^a	3.40	3.66
	%Ptn	3.77	3.08	2.86	2.80	2.76 ^a	2.81 ^a	2.99
	DMI (kg) ^d	16.05	20.38	22.98	23.27 ^a	24.71	24.31	22.66
	NDFI%BW ^e	.88	1.16	1.31	1.34	1.41	1.36	1.26

^aDifferent among treatments within parity at P<0.05.

^bLinear contrast for week effect significant for all variables at P<0.05.

^cLinear and quadratic contrasts for week effect significant for all variables at P<0.01.

^dDaily dry matter intake

^eDaily neutral detergent fiber intake expressed as percentage of body weight

RESEARCH PROCEDURES

RECENT MODIFICATIONS TO THE NEUTRAL DETERGENT FIBER PROCEDURE

D.R. MERTENS

Introduction

Neutral detergent fiber (NDF) is commonly used for routine forage analysis and ration balancing for dairy cows in many parts of the United States. However, many modifications of the method are being used by commercial feed testing and research laboratories. Sodium sulfite was included in the original techniques to assist in removal of nitrogen contamination from NDF. Subsequent research suggested that sulfite reduced the recovery of phenolic acids and yielded lower lignin values when measured after sequential neutral and acid detergent extraction. It was recommended that sulfite be eliminated because sequential lignin was reduced, although it was not determined whether or not sulfite was removing a contaminant of lignin. Recently, ethylene glycol monoethyl ether was banned in Europe because it is a potential mutagen. Triethylene glycol has been recommended as a safe alternative. The objectives of this study were to determine the effects of sodium sulfite and triethylene glycol on NDF values as a continuation of our efforts to establish a standard NDF method for approval by the Association of Official Analytical Chemists.

Materials and Methods

In our current NDF procedure, 45 to 50 mls of neutral detergent (ND) is refluxed with the sample for 30 minutes. Then the remaining 50 to 55 mls of ND is added with 2 mls of an amylase extract and refluxing is continued for an additional 35 minutes. During the transfer of the sample from beaker to fritted-disk crucible a second 2 ml of amylase is used to remove remaining starch. The sample is soaked for 3 to 5 minutes in boiling water and acetone twice each in order; then dried and weighed. The modifications included the addition of .5 ml of sodium sulfite to the initial 45 to 50 ml of ND and the substitution of triethylene glycol for ethylene glycol monoethyl ether (volume/volume) in the ND. In addition, a commercially prepared ND solution containing triethylene glycol was evaluated (Columbus Chemical Industries, Inc., Columbus, WI.).

Results and Discussion

The results listed in Table 1 indicated that sodium sulfite lowers NDF values. This effect is especially true for dried brewers grains and dried distillers grains which were reduced by 12 and 10 percentage units, respectively. These feeds are heat dried suggesting that sulfite is important in removing artifact NDF associated with Maillard (or nonenzymatic browning), products. Analyses of other samples of dried brewers and distillers grains confirm that sulfite results in dramatic reductions in NDF values. The NDF of meat meal was also reduced substantially. The remaining twenty one feeds averaged 1.2 units less NDF when sulfite was included in the method.

When averaged across laboratory and commercially prepared reagents, substituting triethylene glycol for ethylene glycol monoethyl ether had no effect on NDF analyses. The one discrepancy

associated with meat meal was probably related to filtration difficulties. It was observed that triethylene glycol does not have the antifoaming activity of the monoethyl ether; however, this did not effect the procedure significantly.

It was observed that the pH of commercially prepared reagent was 7.8. Since this is higher than the 7.0 recommended for ND, an extra series of analyses were performed after the ND was adjusted to pH 7.0 using hydrochloric acid. The results indicate that the pH of ND solution is critical for proper analysis. Allowing the pH to be 0.8 units higher than neutral resulted in NDF analyses that were 0.4 lower than expected if meat and fish meal are excluded.

Recommendations:

1. Sodium sulfite should be included in routine NDF analysis to remove nitrogen contamination, especially if feeds have been heated.
2. Triethylene glycol can and should be substituted for ethylene glycol monoethyl ether in the neutral detergent reagents.
3. The pH of neutral detergent should be checked and adjusted to pH 7.0 using hydrochloric acid or sodium hydroxide. Solutions should be discarded if not between pH 6.0 and 8.0, initially.

Table 1. Difference in neutral detergent fiber due to sodium sulfite, triethylene glycol and pH.

Feed Ingredient	Reagent source: Treatment: without sulfite	USDFRC ^a with ^c sulfite ^f	USDFRC with ^d triethylene ^g	USDFRC with ^d triethylene ^g	CC1 ^b pH adj. with ^e triethylene ^g	Average with ^e triethylene ^g	CC1 original with ^e triethylene ^g	CC1 pH adjusted ^c difference ^h
	%NDF ⁱ	%NDF	%NDF	%NDF	%NDF	%NDF	%NDF	%ND
Bermuda grass hay	71.07	1.02	0.19	0.12	0.03	-1.31	-1.19	
Bromegrass	69.53	0.26	0.49	0.68	0.58	-0.54	-1.22	
Barley hay	60.75	1.64	0.68	0.03	0.36	0.14	0.11	
Corn silage	39.70	1.42	-0.13	0.20	0.03*	-0.41	-0.61	
Alfalfa hay	48.91	1.29	-0.64	-0.13	-0.38	-0.57	-0.44	
Alfalfa silage, low moisture	48.28	2.17	-0.34	-0.49	-0.42	0.38	0.88	
Ladino clover	35.01	2.03	0.00	0.93	0.47	0.20	-0.73	
Alfalfa leaves	20.73	1.47	-0.33	-0.40	-0.36	-0.66	-0.26	
Com grain	13.84	1.34	-0.04	-0.60	-0.32	-0.88	-0.28	
Ear corn, high moisture	17.28	0.84	0.06	-0.30	-0.12	-0.64	-0.33	
Oat grain	27.83	0.40	-0.34	-0.29	-0.31	-1.06	-0.77	
Wheat grain	13.21	-0.24	-0.53	-0.56	-0.55	-1.18	-0.61	
Barley grain	20.51	-0.74	-0.09	0.07	-0.01	-0.62	-0.69	
Brewers grains, dried	55.85	12.37	0.67	-0.31	0.18	-1.51	-1.20	
Distillers grains, dried	39.40	9.79	1.07	-0.21	0.43	-1.70	-1.49	
Soybean hulls	65.64	0.92	0.68	0.46	0.57	0.45	-0.01	
Beet pulp, dried	39.14	2.63	-0.88	-0.63	-0.75	-1.87	-1.25	
Citrus pulp, dried	22.28	0.46	0.30	0.36	0.33	0.53	0.17	
Wheat middlings	37.23	0.25	0.66	1.04	0.85	-0.08	-1.12	
Soybean meal (44% CP)	16.29	1.70	-0.33	-0.75	-0.54	-0.79	-0.04	
Sunflower meal	40.37	1.68	0.96	-0.21	0.37	0.74	0.96	
Canola meal	24.48	1.89	-0.05	-0.13	-0.09	0.65	0.78	
Meat meal	39.53	6.98	-3.64	-10.98	-7.31	0.96	11.95	
Fish meal	9.30	2.03	-0.41	-0.80	-0.61	1.95	2.76	
AVERAGE (All)	36.50	2.23	-0.08	-0.55	-0.32	-0.32	0.22	
AVERAGE (without meat and fish meal)	37.60-	2.03	0.09	-0.06	0.02	-0.49	-0.42	

^aReagent mixed at the U.S. Dairy Forage Research Center.

^bReagent obtained from Columbus Chemical Industries, Inc., Columbus, WI.

^c.5 gm of sodium sulfite added to the neutral detergent reagent immediately prior to refluxing.

^dTriethylene glycol substituted for ethylene glycol monoethyl ether in the neutral detergent reagent.

^eThe pH was adjusted from 7.8 in the original to 7.0 using hydrochloric acid.

^fDifference from USDFRC without sulfite.

^gDifference from USDFRC with sulfite.

^hDifference between CC1 original and CC1 pH adjusted.

ⁱNeutral detergent fiber as a percentage of dry mater.

COMPARISON OF IN VITRO METHODS FOR MEASURING DIGESTION KINETICS

D.R. MERTENS, D.R. BUXTON, and H.G. JUNG

Introduction

Rate and extent of digestion of plant cell walls is a primary factor limiting intake and digestibility of forages. Progress in studying plant characteristics that influence digestion kinetics is hampered by the lack of a standard technique for measuring rate and extent of digestion. In situ measurements using samples placed in dacron bags that are suspended in the rumen of fistulated cows are affected by the variable ruminal environment within and among cows. In artificial rumens or in vitro techniques, samples are placed in tubes or flasks with buffer solutions and are inoculated with rumen fluid from fistulated animals. In vitro measurements of digestion kinetics may be affected by differences in techniques, especially at early digestion times. Members of the Cell Wall Characterization and Utilization Work Group performed a collaborative study to determine the differences in in vitro measurements among the three current in vitro methods being used.

Materials and Methods

In method A, 0.5 gm samples were incubated with 20 ml of Kansas State buffer and 5 ml of rumen fluid in 50 ml centrifuge tubes capped with rubber stoppers containing one way bunsen valves that allow fermentation gases to escape. 12.5 ml of buffer was added to the sample one hour prior to inoculation and the remaining buffer is combined with the rumen fluid inoculum. Tubes are purged with carbon dioxide prior to inoculation.

In method B, 0.5 gm samples were inoculated with 24 ml of McDougalls buffer and 6 ml of rumen fluid in 50 ml screw cap tubes that are sealed without gas pressure relief valves. Buffer solution was reduced by bubbling carbon dioxide through the solution for at least 3 hours. Inoculum was added to the buffer and this mixture kept under carbon dioxide until it was added to the dry sample in the tubes without purging with carbon dioxide.

In method C, 0.5 gm samples were incubated with 40 ml of Goering and Van Soest buffer and 10 mls of rumen fluid in 125 ml Erlenmeyer flasks with ports connected to a manifold for purging with carbon dioxide. All buffer is added to the samples after the flasks were placed in the water bath. Buffer and samples are reduced by purging with carbon dioxide and injecting 2 ml of a sodium sulfite-cysteine hydrochloride reducing agent. The 10 ml of inoculum was injected into the flasks after the solutions were reduced as indicated by clearing of a reducing indicator.

Method D was similar to C, except the inoculum was prepared by blending approximately 300 ml of rumen solids with 600 ml of rumen liquid for 45 seconds at high speed before filtering through 4 layers of cheesecloth and a 150 mesh plastic cloth. All other methods were inoculated using rumen fluid squeezed through 4 layers of cheesecloth.

Each collaborator prepared the samples and reagents in their laboratory, but all inoculations and incubations were done simultaneously at Madison. Samples were frozen after the specified fermentation period and analyzed for neutral detergent fiber (NDF) at the Madison laboratory. Three alfalfa hay samples containing 55.9, 49.8 and 39.2% NDF were evaluated.

Results and Discussion

All methods appeared to measure the maximal extent of digestion equally as indicated by similar residues remaining after 103 hrs of incubation. Similarly, the amount of NDF solubilized by soaking and refluxing with buffers at 0 hrs of fermentation was not different among treatments. By 6 hrs of fermentation there was a small difference between methods C and D compared to A and B. This difference among methods increased after 24 hrs of fermentation where methods C and D were different from B which was also different from A. By 48 hrs of fermentation the difference among treatments had narrowed so that only treatment A was different from B, C and D. Measurement of rate of digestion is subject to large errors when only four time-observations are used; however, there were differences in the rate of digestion between methods A, B, and C or D. Research is planned to determine the effects of differences in in vitro methods on measuring rates of digestion. The maximum extent of digestion was not affected by in vitro technique and collaborative research efforts are in progress to evaluate factors limiting the maximum extent of digestion of plant cell walls.

Table 1. Comparison of four in vitro methods for measuring rate and extent of digestion.

Time	Method A		Method B		Method C		Method D	
	NDF Residue	Average (Std.Dev.)	NDF Residue	Average (Std.Dev.)	NDF Residue	Average (Std.Dev.)	NDF Residue	Average (Std. Dev.)
Percentage of Sample Dry Matter								
0	43.5	(1.01)			43.8	(0.24)	43.8	(0.61)
6	41.4	(0.48)	41.8	(0.85)	40.5	(0.34)	40.7	(0.33)
24	34.3	(0.85)	30.8	(1.10)	27.5	(0.50)	28.5	(0.38)
48	28.6	(2.15)	25.9	(0.51)	25.4		25.6	(0.20)
103	24.9	(1.00)	24.9	(0.11)	24.6	(0.36)	24.6	(0.54)

USING NEAR INFRARED REFLECTANCE SPECTROSCOPY TO PREDICT NUTRIENT CONCENTRATIONS IN OPEN POPULATIONS

R.G. DADO and D.R. MERTENS

Introduction

Near infrared reflectance spectroscopy (NIRS), as currently used, does not measure chemical composition; instead, it predicts composition using equations developed from a calibration set of feeds. The intensity of reflected light is measured at several hundred wavelengths for each sample and is mathematically transformed. The chemical composition of the calibration samples is regressed statistically against transformed reflected light and an equation is obtained usually using one to nine wavelengths. Increasing the number of wavelengths used in an equation will lower the regression error for calibration, but can add statistical variation when the equation is used for prediction. NIRS has usually been evaluated in research settings where all samples to be predicted by NIRS are available for selection as members of the calibration set (a closed population of samples). However, using NIRS for routine feed testing requires the prediction of samples in open populations which are theoretically infinite in number and are comprised of samples that do not have an opportunity to be

used in calibration. An experiment was designed to use a calibration set to evaluate variables associated with the accuracy of NIRS in open populations. Objectives included testing the hypothesis that frequent recalibration would improve the accuracy of NIRS analysis and determining if a single software program for generating equations or if a specific number of wavelengths would prove most consistent for predicting individual chemical components.

Materials and Methods

Alfalfa silage (AS), corn silage (CS), high moisture ear corn (HMEC), and soybean meal (SBM) samples were collected weekly for approximately one year from several different storage units at the U.S. Dairy Forage Research Center. Samples were dried at 55°C for 24 hours and ground with a 1 mm Wiley mill for chemical analysis and a 1 mm UDY mill for NIRS analyses. Analysis included dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF). Calibration equations were generated via stepwise multiple regression using two software programs. Program "BEST" requires the data from all wavelengths to have the same mathematical transformation while program "CAL" allows the data from each wavelength to have different math transformations. For each feed, component, and program an initial calibration was performed to generate equations with one to four wavelengths. Recalibration was performed four times, each occurring after four weekly samples were added to the base calibration sample set. Consequently, 80 equations for each feed were generated and used to predict nutrient concentrations in 12 validation samples (8 for CS) collected during the last 12 weeks of the experiment.

Results and Discussion

With the exception of one AS-CP sample the range in chemical composition of the validation sets was within the range of the calibration sample sets (base + recalibration sample sets). Ranges in the CP and NDF concentration as a % of DM for the samples used in calibration were AS: 14.6-26.4, 32.0-54.0; CS: 6.6-10.3, 29.7-52.3; HMEC: 7.8-11.0, 9.5-32.6; SBM: 42.2-55.4, 10.2-27.4. The usefulness of frequent recalibration appears questionable (Table 1). Only the first recalibration lowered prediction error significantly and that occurred for only two feeds. No performance differences existed between software programs ($P>0.10$ for all feed x component combinations). The ratio of NIRS to chemical analysis errors averaged 3.26 ± 2.0 . Neither program performed better than the other, nor did the higher-ordered equations outperform the lower-ordered ones consistently (Table 2). The wavelengths selected also varied among recalibration. This variability in calibration equations reflects the statistical approach used for NIRS. In open populations, NIRS calibration methods are needed in which only wavelengths that have a specific chemical-physical relationship are used in prediction equations.

Table 1. Standard error of performance (SEP) for NIRS prediction equations by recalibration.

	Base Calibration Set		Recalibration Set			
	N	SEP	1	2	3	4
AS	80	1.74	1.65	1.67	1.68	1.62
HMEC	33	0.65	0.87	0.80	0.66	0.55
SBM	28	1.65 ^a	1.23 ^b	1.16 ^b	1.17 ^b	1.19 ^b
CS-CP	35	0.55	0.40	0.41	0.40	0.70
CS-NDF	35	2.78 ^a	1.99 ^b	1.77 ^b	1.33 ^b	1.76

^{a,b} Means in same row with different letters differ at P<0.05. Other means P>0.20.

^c Average SEP of the "BEST" and "CAL" equations which had the lowest SEP from among the equations with 1-4 terms.

Table 2. Equations providing the lowest standard error of performance for each feed, component, and recalibration.

Recalibration Set	AS		CS		HMEC		SBM	
	CP	NDF	CP	NDF	CP	NP	CP	NDF
Base Cal. Set	C1	B3	C1	C3	C1	C3	C1	C3
1	C2	B3	C1	B4	B1	B3	B2	B1
2	B4	C3	C2	B3	B2	C2	B2	C2
3	B4	C3	C2	C1	B3	B3	B3	B3
4	C4	C3	C2	B4	C2	B4	C4	B4

C, B - "CAL" or "BEST" software programs.

1, 2, 3, 4 - # of wavelengths utilized in the equation.

EVALUATION OF A DIFFUSION TECHNIQUE FOR PREPARING ¹⁵N SAMPLES FOR ANALYSIS

J.A. LORY and M.P. RUSSELLE

Introduction

¹⁵N is a heavy non-radioactive isotope of the element nitrogen. It is commonly used as a tracer for N in agricultural systems. To prepare ¹⁵N samples for analysis they must be concentrated, typically by distillation. The primary problem in concentrating ¹⁵N samples is cross-contamination.

An ammonia diffusion technique described by P.D. Brooks et al. (Agron. Abstr., p. 179, 1987) avoids cross-contamination problems. This method captures the sample on an acid impregnated glass fiber disk in a disposable container. We evaluated the method's ability to prepare low N, high volume samples for ¹⁵N analysis.

Materials and Methods

All samples were diffused in 140-mL disposable polypropylene containers with screw top lids. A glass fiber filter disk (Fischer GF/D) formed with a paper punch was impaled with an acid washed stainless wire (#308, 0.6 mm). The stainless steel wire was wedged in the top of the cup suspending the disk over a simulated Kjeldahl digest sample. 5 M H₂SO₄ was then applied to the disk. 10 M NaOH was slowly poured down the side of the container raising the sample pH to >13. The top was quickly applied and firmly tightened. The high pH of the sample drives NH₄⁺-N to the volatile NH₃-N form. Ammonia that diffuses to the acid-impregnated disk is converted back to NH₄⁺-N, concentrating it on the disk for analysis. Special care was taken to insure sufficient H₂SO₄ was applied to the disk to capture all N in the sample.

Two separate experiments were performed. The objective of the first experiment was to determine if sample N content and sample atom % ¹⁵N affected samples prepared for analysis by diffusion. Six ¹⁵N concentrations (0.1996 to 0.9826 atom % ¹⁵N) were evaluated at 6 NH₄⁺-N concentrations (100 to 3000 μ g). Final volume of all samples was 50 mL. There was no active mixing or swirling of the samples during the 14-day diffusion period. The isotope ratio of the N recovered on the disk was compared to the isotope ratio of the initial solution. The experiment was run twice.

The objective of the second experiment was to quantify alternative N "sinks" other than the acid impregnated disk in the diffusion system. Pools of N quantified were: N trapped on the disk, N remaining in the sample solution, N remaining on the sides of the cup, N on the top of the cup, and N on the wire. We also evaluated the effect of carefully washing down the sides of the cup by "swirling" after 24 hours on the predictability of N recovery on the disk. Swirled samples were tipped and slowly rotated exposing the sides to the high pH solution. Samples (1000 μ g N) were prepared and diffused as described in experiment 1 for 14 days.

Results and Discussion

Results of experiment 1 demonstrated that atom % ¹⁵N of N recovered on the disk was inaccurate in low mass samples (Figure 1). Diffused samples also had high C.V.'s due in part to incomplete recovery of N on the disk.

In the second experiment we added the modification of swirling with the expectation of increasing N-recovery on the disk. Results of experiment 2 are summarized in Table 1. There was incomplete recovery of N on the disk with both methods ($p < 0.05$). Swirling increased the recovery of N on the disk from 90.8 % to 98.4 % and reduced variability by 66%. Swirling reduced the amount of N remaining on the sides of the cup by 97% but had no effect on N recovered on the top and wire. A small amount of N remained in solution after diffusion.

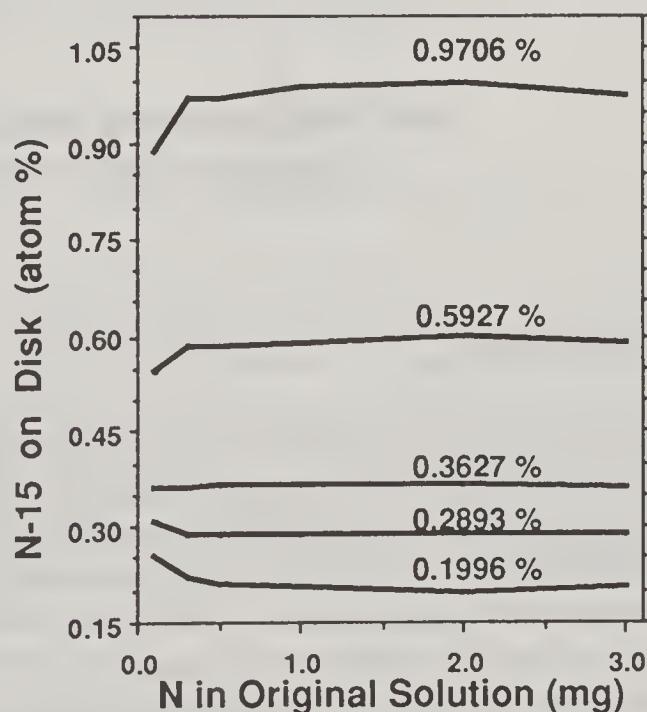


Figure 1. Atom % ¹⁵N recovered on the disk as a function of sample mass. Each line represents a different initial atom % ¹⁵N in the sample solution.

We observed significant moisture buildup on the sides and top of the cup at times during the 14-day diffusion, especially immediately after adding NaOH to the sample. Adding the base to the strongly acidic digest generated heat. Water condensed as warm moist air in the cup contacted the cooler sides and top. While condensate existed on the sides and top, it served as an alternative sink for diffused N. As the water evaporated from the sides and top, the N could be held on the plastic surface by forming salts with anion contaminants from the sample, adsorbing to dust contaminants, or bonding to impurities in the sample. Rinsing down the sides with the high pH solution reduced or eliminated N held on the sides by these mechanisms.

We do not recommend this method for high volume (50 mL) low-N (<1000 μ g) samples. Before adopting this method for any volume and amount of N, thoroughly evaluate the method for the specific sample type and laboratory conditions.

Table 1. Quantification of pools of N after 14 days diffusion. Swirled samples were carefully tipped to rinse the sides with sample solution after 24 hours.

Pool	Mean (se)	Range	n
Disk			
nonswirled	908 (9)	827-968	23
swirled	984 (3)	975-996	10
Sides			
nonswirled	69 (7)	17-152	24
swirled	2 (1)	1-10	10
Solution*			
	3 (0.5)	3-4	3
Top			
	4 (0.6)	0-12	28
Wire			
	2 (0.8)	0-14	17

New Methodology for the Characterization of Lignins: Silylation and $^{29}\text{Si}/^{13}\text{C}$ NMR - Model Studies.

J. RALPH

Introduction

Lignin is a difficult polymer to chemically characterize even in favorable materials such as woods. With grasses, and more especially legumes, the literature is rather sparse. We intend to complete a detailed characterization of forage lignins by traditional chemical as well as modern spectroscopic means. With this knowledge, and by coming to understand the interactions between components in the cell wall of fibrous plants, we hope to unravel the complex issues of forage digesti-



bility by ruminants. (It has become clear that there is a limit to which simple quantification of cell wall components can aid in a comprehension of the key factors limiting digestion).

The most powerful method presently for obtaining a detailed structural picture of lignin is ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Indeed, NMR is an essential tool in any chemical research, particularly involving organic compounds.

¹³C NMR

In simple molecules, ^{13}C NMR is valuable in that a spectrum yields one peak for each carbon in the molecule. The position of that peak is influenced primarily by the environment of the carbon within the molecule. Using simple NMR experiments it is possible to elucidate how many protons (H-atoms) are attached to each carbon, and a complete structural picture can be built up. Indeed, chemists routinely identify complete unknowns, solely by NMR experiments, in as little as 5 minutes. Complex molecules draw chemists into increasingly sophisticated multiple pulse experiments to reveal complete structural information. These days it is possible to completely sequence and assign complex proteins of even several hundred thousand molecular weight, and NMR will also provide a 3D structure because it is capable of revealing atoms that are near each other in space.

Polymeric lignin presents more of a challenge. It is a rather poorly defined three dimensional polymer having a substantial molecular weight range, and is irregular in the sense that it has no uniformly repeating units. However, it is formed from up to 3 simple precursors and does contain a relatively small number of discrete and identifiable dimeric units, Figure 1. ^{13}C NMR has had a powerful impact on the structural characterization of this polymer, and it is probably true to say that a single ^{13}C NMR spectrum today yields all the information that was gleaned from 50 or more years of painstaking research. The power of ^{13}C NMR as an NMR method is attributed to the low natural linewidths and high dispersion of ^{13}C resonances, and the sensitivity of the ^{13}C nucleus to even complex polymers give very detailed spectra. The additional fact that the position (or chemical) assignments in the polymer can be compared to those of smaller model compound. This means that, while the assignments in the polymer can be compared to those of small molecules. The advantage here is that the size, and because of their relative simplicity, it is possible to make spectral assignments (for all peaks in the spectrum) with a reasonable degree of confidence.

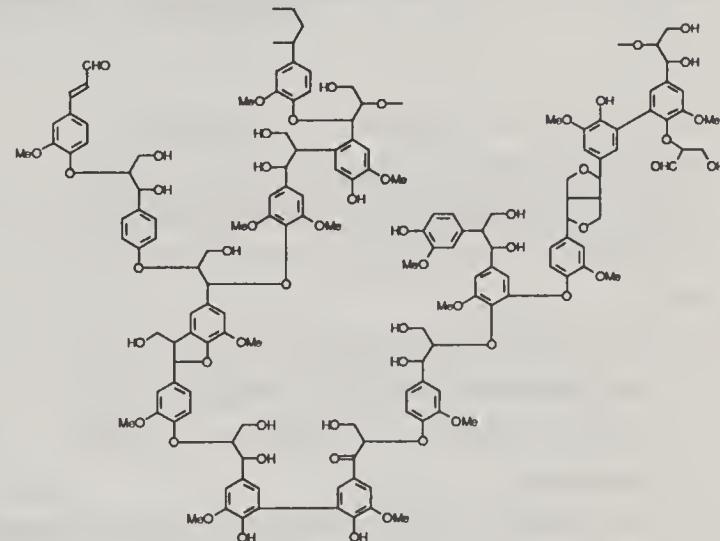


Figure 1. Adler's representation of lignin

NMR of Other Nuclei

The NMR method is not restricted to proton and carbon. Nearly every element in the periodic table has at least one NMR-active isotope. Lignin in its unmodified form however contains only C, H,

and O. As ^{17}O NMR is completely useless for polymers because of the huge natural linewidths (which increase roughly with molecular weight) the possibilities for NMR of lignins are confined to proton and carbon unless the lignins are modified. However, rather than being a drawback, it is routinely desirable to derivatize lignin. For example, acetylation is frequently used to improve the solubility of lignins in organic solvents (particularly chloroform and acetone) and give an additional handle on the OH functionality.

^{29}Si NMR

Initial studies on the trimethylsilylation of lignins, with the aim of characterizing these modified lignins by ^{29}Si as well as ^{13}C NMR, showed that; the derivatization was easily carried out, the products (from milled lignins) were very soluble in common organic solvents such as chloroform, acetone and even hexane, and that ^{29}Si NMR spectra appeared to show promising detail and very good sensitivity when using polarization transfer (PT) techniques (e.g. INEPT). However, the products (both from model compounds and lignins themselves) were unstable to degradation and polymerization.

Presently we are studying the silylation of lignin and model compounds using, initially, tert-butyldimethylsilyl (TBDMS) derivatives. The reasons for the choice of this derivative are:

1. the significant amount of literature on the successful use of this derivative in synthetic chemistry for hydroxyl group protection.
2. the stability of the alcohol and phenol derivatives to storage, heat, hydrolysis, chromatography, and even oxidation and hydride reduction.
3. the observation that the polarization transfer enhancements of the silicon signals in ^{29}Si NMR via INEPT or DEPT are higher. This greater experimental sensitivity means that less time is required to achieve a spectrum of a given signal to noise.
4. the ability to selectively functionalize, or selectively remove silyl functionality from, aliphatic vs phenolic OH groups.

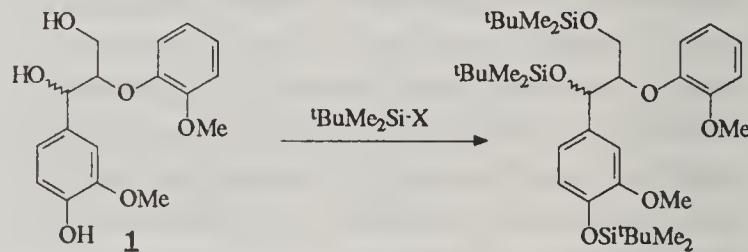


Figure 2. TBDSIylation of lignin models.

Lignin model compounds, such as the β -aryl ether model 1, figure 2, are synthesized. Silylation of these models, figure 2, and determination of their NMR spectra, is providing the necessary database for interpretation of spectra of the lignin polymer. ^{29}Si NMR, like ^{13}C NMR, allows the chemical shifts in the polymer to be ascertained from the shifts in well-chosen model compounds. Complete and unambiguous ^1H , ^{13}C , and ^{29}Si NMR assignments are being accomplished by 2D ^{13}C - ^1H (short- and long-range) correlation experiments, ^{29}Si - ^1H correlation (figure 3), and others.

Impact

The completion of the first stage of this project will provide a further powerful weapon in the chemists' arsenal to elucidate details of cell wall structures. The usefulness of the technique is obviously not limited to the study of forages, but should help us substantially in this difficult area.

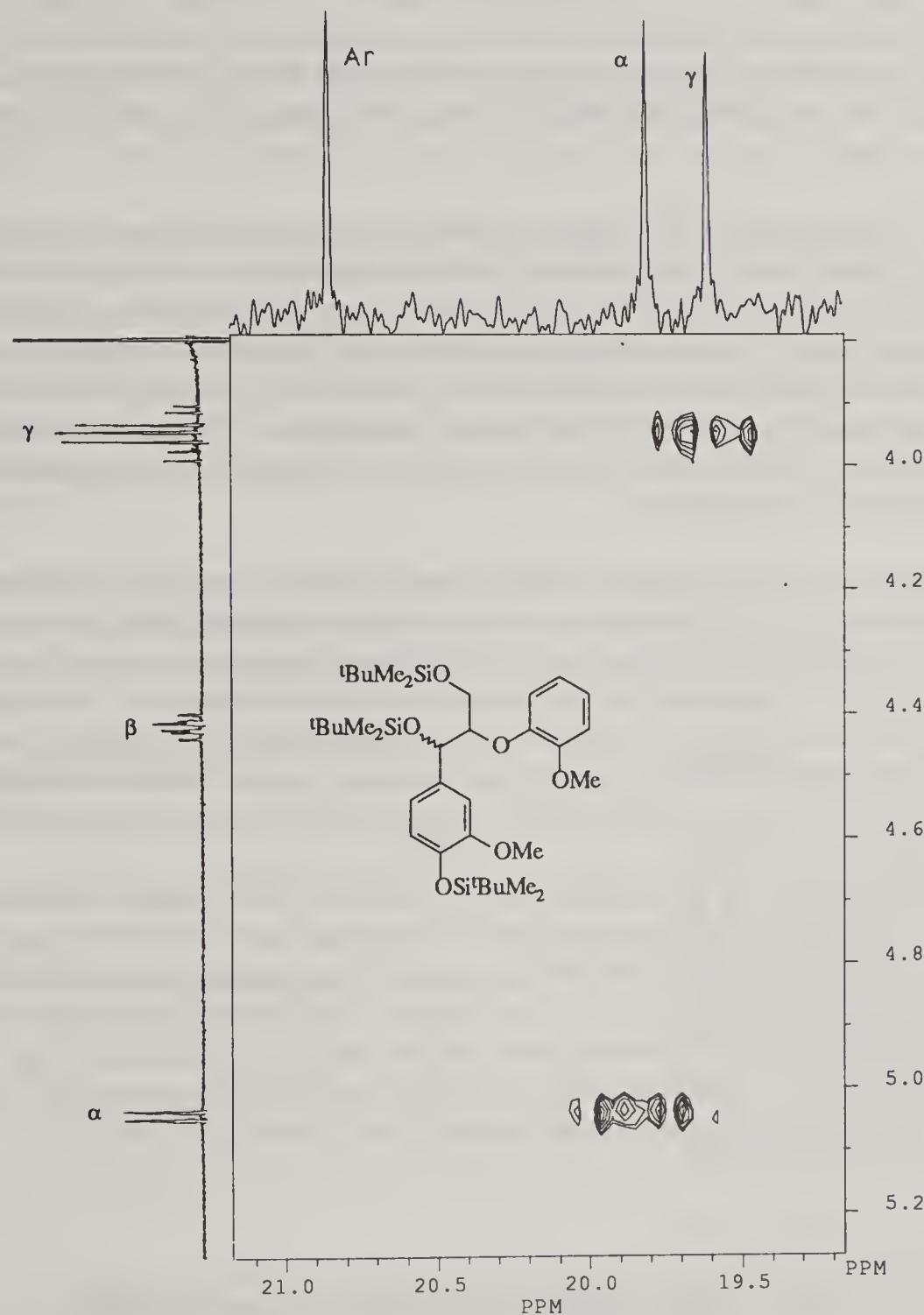


Figure 3. Si-H correlation.

FORAGE UTILIZATION BY CATTLE AND SHEEP

U.S. DAIRY FORAGE RESEARCH CENTER ANNUAL DAIRY OPERATIONS REPORT, FEBRUARY 1989

L. L. STROZINSKI

The research center herd has now reached its full capacity. Present total herd count is 552 (266 cows and 286 herd replacements). At the present time we are milking 243 cows averaging 59 pounds of milk per day. Our DHIA rolling herd average has risen from 16,397 pounds of milk, 595 pounds of fat, 507 pounds of protein to 17,585 pounds of milk, 648 pounds of fat and 539 pounds of protein during the past year.

I feel that the overall quality of the herd has continued to increase. Six cows in the herd are currently on the Holstein Association list of top 10,000 type-production index cows in the country. The reproductive status of our herd continues to be good. At present, average days open for the herd is 109 days. Average age at freshening for first calf heifers is 24 months. Therefore, we continue to have a large number of quality herd replacements. Calf mortality continues to be very low with a loss of only 4 out of 170 female calves born during the last year. This year eight registered bred heifers were sold to the Southern Illinois University. Marketing and culling strategies for surplus dairy stock continue to be developed.

During the past year 12 major feeding trials have been conducted involving more than 370 animals. The major project in the dairy operation at the present time is the remodeling of the milking parlor. We are changing from the present four on a side - side opening system to a double six herringbone design. The new parlor equipment will be Boumatic from Dairy Equipment Company of Madison. It will feature their completely automated and computerized animal I.D. and milk measurement system which will integrate with our present herd computer system. In addition to improved milking and data collecting, we hope to improve parlor throughput and overall labor efficiency.

The center's manure handling and bedding system continues to be a problem, however progress is being made. A new manure separator designed by Dr. Koegel and his staff was tested at the farm this year and is now installed in the manure separation room. Some problems still surface but the unit looks very promising. Dr. Koegel is in the process of building yet another "new and improved" version of the separator. Outside interest in this manure separator design is growing. As in past years, the center continues to host many visitors from around the world. This summer we will cooperatively host the Wisconsin State Forage Council field day on June 20.

U.S. DAIRY FORAGE RESEARCH CENTER ANNUAL FIELD OPERATIONS REPORT, FEBRUARY 1988

B.C. VENUTO

For crop managers and farmers the 1988 season was dominated by the dry weather. For many of us 1988 was the driest crop season of our professional lives and most of us hope that it will remain so.

It was not, however, unique to this century as evidenced by a quote from the Secretary of Agriculture's report to the President in 1936. "This year's drought, besides causing enormous damage to crops, inflicted great hardship on farm people throughout an immense area, particularly in states that had not recovered from the drought of 1934. It aroused fears that our climate might be undergoing a permanent change, though there is no scientific evidence that such is the case, and led to speculation as to whether recent conditions might possibly be due to some human activity".

Meeting the Challenge of 1988

The objectives of 1988 were not unlike those of any other year. Our primary goals were to meet the forage requirements of our research program and produce quality feed for the dairy herd. We were frustrated in these efforts due to the dry weather but we were also challenged. We were forced to think creatively, reconsider old practices and consider alternative crop strategies.

As was certainly the case for many dairy farmers in Wisconsin, our forage and grain yields were much below normal (Table 1). To compensate for reduced forage yields a much greater percentage of our corn was harvested as silage with needed corn for grain being purchased. Sudex was planted in early July on older alfalfa fields that had in some cases yielded only 4 bales of second crop. Roadsides and waste areas were mowed and baled for dry cow and heifer feed. Corn stalks were chopped and ensiled after combining high moisture ear corn. A riparian irrigation permit was obtained from the Wisconsin Department of Natural Resources and an attempt was made to irrigate 80 acres of alfalfa.

In summary the 1988 crop season was long, hot, hard and frustrating. The cooperative effort and positive response of our field crew and staff enabled us to weather the storms (or lack thereof) and achieve our goals. We are all looking forward to a return of normal weather patterns in 1989.

Table 1. U.S. DAIRY FORAGE RESEARCH CENTER
1988 CROPS & YIELDS

Crop	Acres	Yield/Acre	Yield % of Normal
Corn			
Grain	0		
H.M. Ear	83	3.6 Tons (wet)	70%
Silage	149	7.5 Tons (wet)	50%
Winter Wheat	60	62.4 Bushels	80%
Alfalfa	600	2.8 Tons D.M.	60%

CORN

Corn was much below normal in yield. In addition to drought damage about 40 acres suffered severe deer damage. The poorest corn was harvested as silage with some fields yielding less than 3 tons of wet silage per acre.

WINTER WHEAT

Wheat yields exceeded our highest expectations. We had an excellent 87/88 winter with good snow cover and excellent spring soil moisture. Apparently the soil moisture levels were enough to provide a respectable crop.

ALFALFA

First crop yields were 75-80% of normal. Second crop was a disaster. Third and subsequent crops were respectable but account for a small percentage of our totals.

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